Drew University

College of Liberal Arts

Discovery of novel isoindole antibacterial compounds

A Thesis in Chemistry

By

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Abstract

Antibacterial resistance continues to rise as the use of antimicrobial compounds selects for resistant strains to survive and repopulate. By the year 2050, the leading cause of death in the world is projected to be antimicrobial infections causing 10 million deaths per year. The drug discovery process from start to finish averages 10 years in length and 2.6 billion dollars in investment. New antibiotics are generally held in reserve to reduce the chance of resistance to develop. This results in a lack of monetary incentive, which discourages large pharmaceutical companies from investing and leaves the role of development of new antimicrobial compounds to academic research laboratories and small biotech companies. Novel isoindole compounds synthesized at Drew are being optimized for antibacterial potency using a five step synthesis. The compounds are designed based on inhibition of the putative target of the isoindoles, FtsZ, a protein critical to bacterial cytokinesis. Synthesis of a key intermediate compound 4, 1-(4-bromobenzyl)-1 -(4-chlorophenyl)-3-ethoxy-1 H-isoindole, leads to a compound that can be reacted in 1 step to vary the amine side chain at the 3-position (R2) in order to explore the antibacterial potency of the final product. The aminopropyl side chain resulted in the highest potency against *Staphylococcus aureus* likely due to better chelation to the Ca^{2+} located in the interdomain cleft of the FtsZ protein.

Introduction

Chapter 1: General Background

Antimicrobial resistance has received attention from the scientific community for over three quarters of a century, yet problems remain a large concern in the public eye today. The CDC reports in 2019 that more than 2.8 million antibiotic resistant infections occurred within the United States and over 35,000 cases resulted in death.[1] With such a large number of cases, unfortunately, many large pharmaceutical companies are not investing in the discovery and development of new antibiotic agents to combat this large problem due to the lack of profitability with these products.[2,3] With money being invested into research and very few marketable antibiotics being approved, companies eventually decided to shift focus to other diseases.[3] It is important to understand pharmaceutical companies rely on the sale of products to stay in business. While antibacterial research is important, sales ultimately keep these companies in business.[3] Without the help of large pharmaceutical giants, the importance of academic research becomes more prominent.

Bacterial populations evolve overtime through mutations in DNA which provide a biological advantage. However it is important to note that these mutations arise randomly. When DNA undergoes replication, DNA polymerase adds complementary base pairs to produce a complementary strand.[4] As DNA polymerase assembles DNA, errors in base pairing occur. An error in pairing results in daughter DNA having a different sequence of nucleic acids which eventually lead to the production of an altered protein.[4] These changes are known as mutations, where the majority of mutations have no effect, some can cause cell death, and some provide enhanced survivability.[4] Members of the bacterial community with beneficial mutations are more likely to replicate and survive thus causing evolution in the population.

Before continuing further, it is important to define what antibiotic resistance is, and how it differs from persistence. Antibiotic resistant microorganisms have mutations that allow for survival in the presence of an antibiotic compound commonly used to inhibit growth of that species. Resistant microorganisms are able to replicate cells containing this resistance mutation, that allows for a population of cells to survive in the presence of an antibiotic. This is a key distinction from a persistent cell, which are able to survive in the presence of an antibiotic but are unable to produce daughter cells that also survive in the presence of antibiotics. For example, a persistent bacterial infection treated with an antibiotic will kill 99% of pathogenic cells. The surviving bacterial cells will regrow into a new infection where the same antibiotic is able to inhibit the growth of 99% of the bacterial cells. The cycle will repeat where the new daughter cells will be vulnerable to the antibiotic but the same cells that survived previously still are able to survive again (Figure 1). This is a drastically different issue from antibiotic resistant bacterial cells where the initial infection can be treated with an antibiotic that inhibits the growth of 99% of the cells, yet when the surviving cells replicate the same antibiotic will be ineffective at inhibiting any of the new infection.[5]

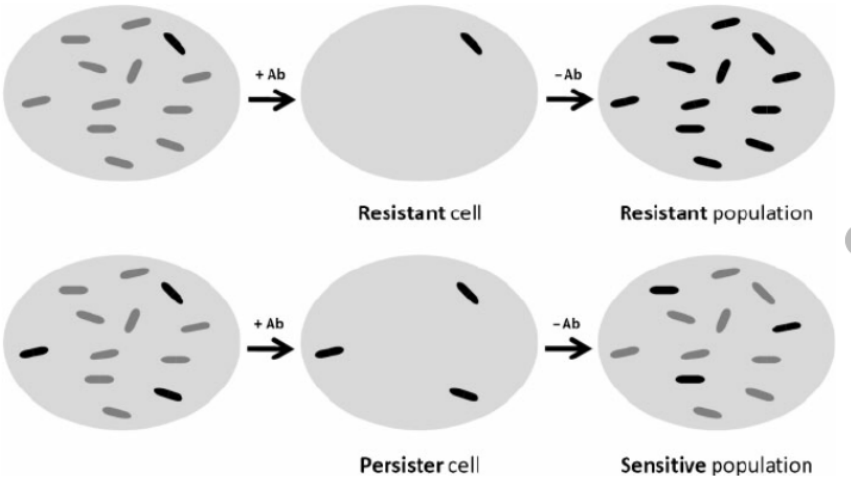


Figure 1: Demonstration of resistant vs persister cell populations. Resistance leads to a larger issue as the new population is unable to be treated with the same antibiotic whereas the persister population is still largely sensitive to the antibiotic. Rewritten with permission[5]

Resistance and persistence occurs at different levels depending on the sensitivity of the antibiotic present. Resistance can occur gradually where a drug of a certain dose may become ineffective, however by increasing the dose these cells may still be killed or have growth inhibited.[6] These bacterial strains can be classified as partially resistant to a drug since a dose dependency occurs. For example, a drug may work at a dose of 10 µg. Eventually, a random mutation will allow for bacterial cells to no longer be inhibited/killed at 10 µg and a 100 µg dose would be required to disrupt activity. This is known as breaking through a susceptibility point, and a larger dose is required.[6] This becomes an issue when a drug is required in such a large dose to treat an infection that it becomes too toxic for the patient. Resistant groups of bacteria are selected over time as we introduce antibacterial agents into our environment. Random mutations occur quickly within microorganism colonies as they replicate rapidly as compared to larger multicellular organisms. The doubling time for microorganisms is generally 4 to 20 minutes.[4]

This gives many opportunities for DNA to have random mutations. As we use antibacterial agents, the cells without any resistance are inhibited while some mutated cells survive.[3] What can be very troublesome is when strains begin to develop multiple resistant traits. For example, a bacterial cell colony can be treated with penicillin, a common antibiotic used frequently to disrupt cell wall synthesis. Eventually resistant cells survive due to random mutations and the selection process. These resistant strains can then be treated with a different antibiotic with a different mode of action, for instance ciprofloxacin that inhibits DNA replication. However over time a resistance mutation to this antibiotic will occur, leaving a strain that is resistant to both of these antibiotic agents and thus requiring a new compound in order to treat this infection.[3]

Methicillin resistant *Staphylococcus aureus* (MRSA) is a largely known antibiotic resistant bacterial strain that has been causing infections around the world.[7] The CDC estimates that over 300,000 infections occur each year with over 10,000 of these cases being lethal.[7] MRSA infections are most commonly spread in hospitals where infected individuals are in close contact with others. This strain of bacteria causes symptoms such as skin irritation, fever, and fatigue. The infection can spread to important organs such as the heart valves, bones, joints, or lungs and lead to death.[8] The resistant strain is unable to be treated with penicillin derivatives since the strain has evolved to be able to survive in the presence of β-lactam antibiotics. β-lactam rings are an important chemical structure present in many antibiotic compounds. General treatment requires the use of antibiotics such as trimethoprim, sulfamethoxazole, clindamycin, minocycline, linezolid, and doxycycline.[8] Even though we currently have other drugs that can treat MRSA, it is important to recognize that the resistance to methicillin can easily arise to these other treatments overtime. An infection resistant to all known antibiotics would be untreatable.

Staying ahead of anticipated multidrug resistant bacteria is important in preventing an infection untreatable by modern medicine.

Resistant cells are able to transfer advantageous genes through a process known as horizontal gene transfer.[9] Horizontal gene transfer allows cells to transfer genetic material to neighboring cells. Conjugation, viral transduction, and plasmids are three main methods in which horizontal gene transfer can occur. Conjugation is where bacterial cells produce a structure known as the pilus which binds to neighboring cells transferring genetic information directly. Viral transduction occurs when a viral vector transfers DNA between neighboring cells.[10] Plasmids are small fragments of DNA that can diffuse into neighboring cells. The genetic material is able to be transferred into neighboring cells allowing for transcription and translation to occur.

The CDC classifies known pathogenic antimicrobial resistant infections on their website with different threat levels from "urgent" to "watch" list.[11] These cases are emerging threats of possible rising diseases. While not all of these cases are related directly to antibiotic resistant strains, a substantial amount are a result of resistance strains becoming more numerous in patients. Examining these cases, we can observe how much of a burden this problem has become on our healthcare system in both the scale of deaths per year as well as the financial strain of treating resistant infections.[11]

One urgent threat listed is *Candida auris*, a multidrug resistant pathogen. *C. auris* is a fungal infection that was first discovered in 2009 from Asia.[12] While this is important to note that this is a fungal infection and not a bacterial infection, the consequence of multidrug resistance is demonstrated here very clearly and parallels multidrug resistant bacterial infections. In just a short amount of time, cases have spread around the world as prevalence rises. Infections from

this pathogen can cause issues in the bloodstream and brain that can lead to death. It is commonly spread in hospitals and long-term care facilities putting already immunocompromised patients at greatest risk of exposure.[12] What makes *C. auris* a large problem is the prevalence of resistance to antifungal agents. The CDC estimates that 90% of cases are resistant to at least one antifungal agent.[12]

Chapter 2: Mechanisms of Antibiotic Resistance

Microorganisms exposed to toxic compounds are always subject to random mutations that may provide enhanced survivability in their environment. Organisms are able to develop resistance through a few common mechanisms. There are four main mechanisms (Figure 2) most commonly seen in resistant cells.[13]

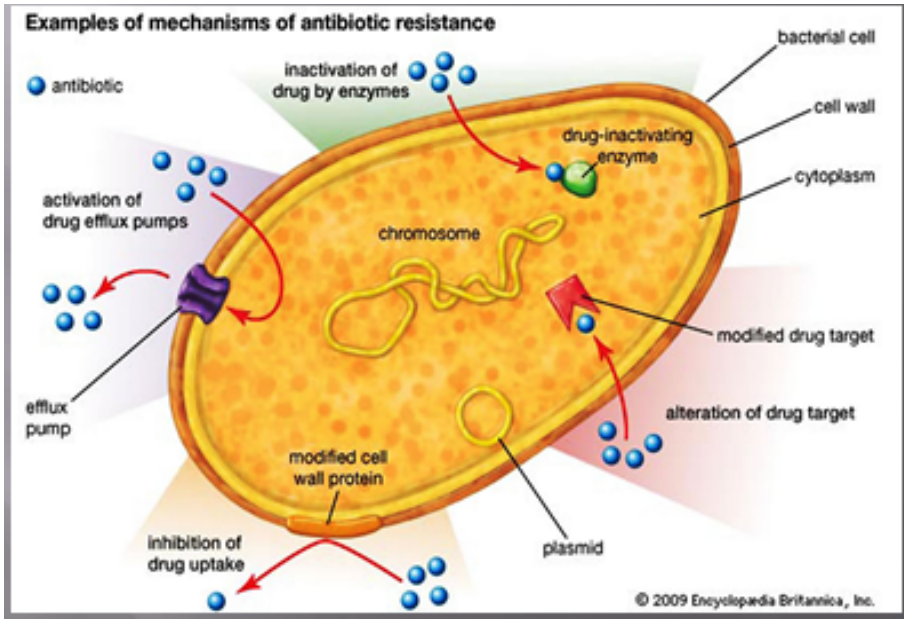


Figure 2: Above is an illustration of common mechanisms that cells are able to become resistant to toxic substances in their environment. This encompasses a large majority of how pathogenic cells become resistant to pharmaceutical compounds. Rewritten with permission[13].

The first resistance mechanism is a mutation that leads to a large increase of efflux pumps. Efflux pumps allow resistant cells to transport toxic compounds out of the cell at a faster rate than the compound can enter the cell.[13] All bacterial cells contain efflux pumps for survival, however resistant strains have been shown to upregulate the number of efflux pumps.[14] Without sufficient compound concentration in the cell, the compound will be ineffective at disrupting biological activity to treat the infection. An example of a case where an increase in efflux pumps are observed is in resistant *S. aureus.* These mutant cells have been shown to have extra NorA, Nor B, NorC, and MDR pumps that all have the ability to transport antibacterial compounds out of the cell.[15]

A different method of inhibition commonly observed in resistant pathogens are drug inactivating enzymes. These cells have enzymes that bind specifically to the drug or toxin and destabilize the molecule in some way that prevents function.[13] These enzymes can either metabolize compounds such as pyrazinamidase or monooxygenase, or can modify the drug molecule itself such as hydrolase or redox enzymes.[16] An example of inactivating enzymes are β-lactamases, found in many Gram-negative organisms such as *Escherichia coli* and *Klebsiella pneumonia*. β-lactamase enzymes are able to inhibit β-lactam antibiotic compounds such as penicillin, cephalosporins, and monobactams.[17] The mechanism of inhibition works by a catalytic enzyme-substrate covalent complex that ultimately catalyzes hydrolysis of many β-lactam antibiotic compounds (Figure 3).[17]

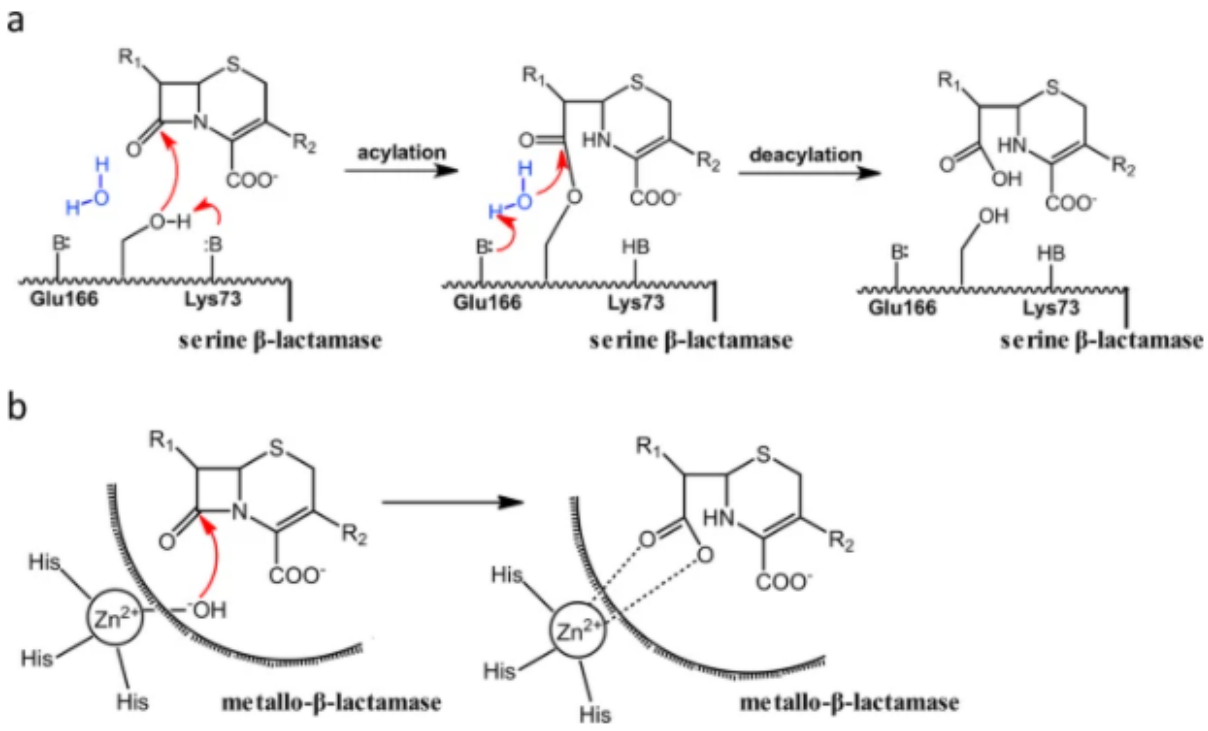


Figure 3: Mechanism for the inhibition of β-lactam ring demonstrates how the four membered ring β-lactam is opened by interacting with glu166 and lys73 of the β-lactamase enzymes. In part b of this figure a metallo based inhibitor demonstrates the chelation of a positively charged metal with a hydroxyl group to open up the ring. Rewritten with permission[17]

A third mechanism of inhibition is modification of a drug target. Erm genes code for an enzyme that modifies the structure of ribosomes within antimicrobial cells resulting in resistance to erythromycin.[18] By modifying the structure of the ribosome via methylation of certain amino acids within the amino acid sequence, the ability of erythromycin to bind to the ribosome is inhibited making the drug ineffective. Examples of pharmaceutical drugs that work by inhibiting

the ribosome are aminoglycosides and macrolides (Figure 4). These antibacterials are no longer effective against modified ribosomes since they lose their ability to bind to ribosomal subunits.[19]

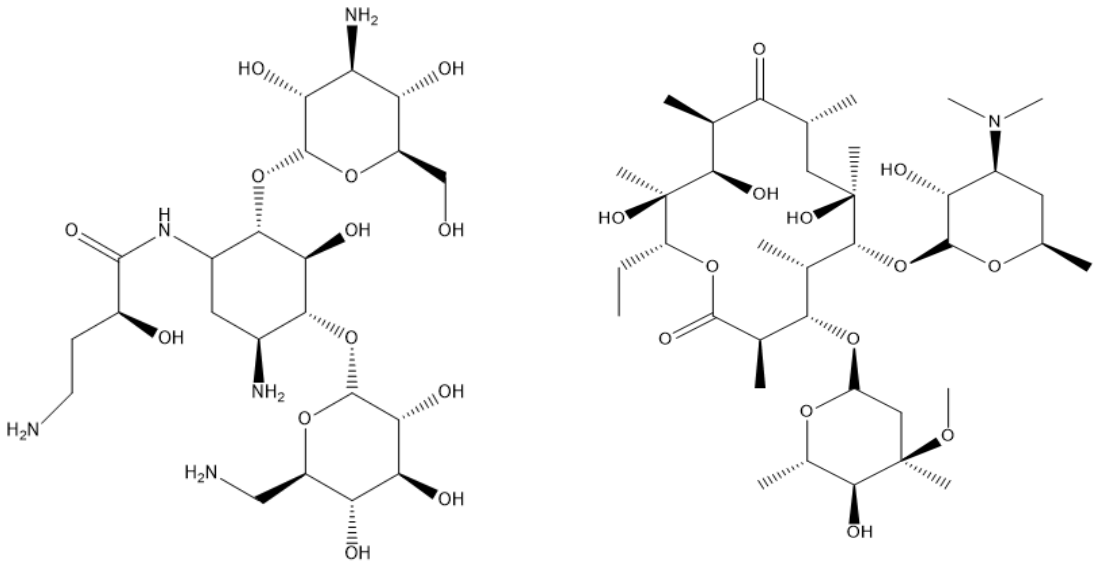


Figure 4: On the left is Amikacin, an aminoglycoside that is an injectable treatment to treat serious bacterial infections.[19] Pictured on the right is erythromycin, a macrolide taken orally to treat a wide variety of bacterial infections.[18]

The final common method of resistance is the cell wall.[13] By preventing the entrance of a drug into the cell, there is no way for the drug to inhibit any biological activity. Many Gram-negative bacteria have this ability to limit antimicrobial compounds from entering cells (Figure 5). The presence of an additional outer membrane as well as the presence of lipopolysaccharides add an additional barrier to the cell. These microorganisms have highly

lipophilic cell membranes, which prevents hydrophilic compounds such as glycopeptides and colistin from entering the cell.[20]

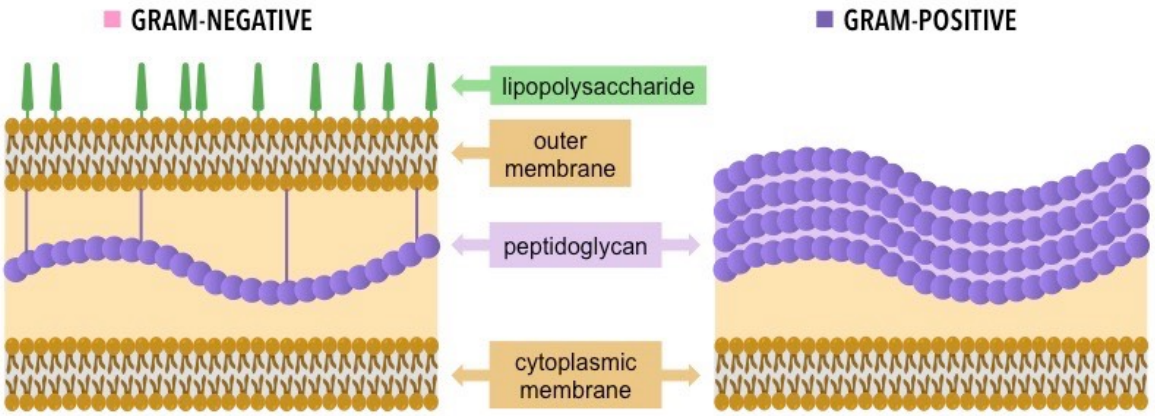


Figure 5: On the left is the structure of the cell membrane in a Gram-negative bacteria vs on the right the structure of a Gram-positive bacteria. Rewritten with permission[21]

Multi-resistant cells can acquire multiple mechanisms listed above in order to be resistant to many different types of drugs on the market. A serious threat that has emerged globally is a group of organisms known as ESKAPE, encompassing *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter* species.[22] All of these organisms are classified as multidrug resistant strains, meaning that they have known resistance to at least three different commonly used antibiotics. Resistance has led to an increase in global deaths from these infections due to treatment failures. The cost of resistant infections in the United States is estimated to be 35,000 lives lost yearly.[1] However, hospitals do have treatments at their disposal to attempt to kill resistant strains. Many

hospitals have antibacterial compounds that are only used in emergency situations. For example, primaxin is an antibiotic compound with limited resistance.[23]

Many hospitals have primaxin in reserve, however they are not prescribed to patients unless other antibiotic compounds fail first to treat infection. These antibacterial compounds are held in reserve because the frequent use will lead to selection of resistant strains. By delaying the use of unnecessary antibiotic treatments, random mutations in pathogenic cells provide no added survivability. Since there is no advantageous effect for mutated cells, they will not dominate the bacterial population. It is extremely important to continue to develop novel antibacterial compounds as eventually random mutations will lead bacteria to become resistant to current drugs. Untreatable strains emerge as dominant infections causing a severe public health crisis.[23]

Chapter 3: Natural and Synthetic Compounds

Bacterial cells exist in an environment constantly in competition with other organisms. Many antibacterial compounds are naturally produced by organisms as defense mechanisms.[24] Humans are not the only organism that can be infected by bacterial pathogens, as other animals and plants deal with the same scenarios. Some of these organisms produce natural compounds that are able to inhibit the growth of bacteria, such as cephalosporins, produced by the fungus, *Acremonium chrysogenum*.[24] Additionally, bacteria also compete with each other for nutrients and resources and have their own mechanisms and compounds of inhibiting other bacterial cells in order to compete for survival.[25]

Natural compounds already exist to kill and inhibit the growth of bacterial cells, which provide a great foundation for the discovery of antibiotic compounds. One example would be cranberry extract.[26] A study found that cranberry extract was able to inhibit the growth of

Porphyromas gingivalis and *Fusobacterium nucleatum.* Compounds within the extract are toxic to certain bacterial cells and thus are a natural antibiotic. This is just one of many examples of the natural defense mechanisms or organisms producing antibiotic compounds in order to protect themselves against bacterial pathogens.[26]

Synthetic analogues of naturally occurring compounds is another common way for antibiotic compounds to be discovered. An analogue is a compound that is similar, but with some structural modification. The objective is to modify a natural compound to improve potency against a resistant strain or to improve pharmacokinetics.. An example of this approach is the development of analogues of penicillin.

Penicillin is one of the most groundbreaking medical discoveries (Figure 6). It is now commonly prescribed around the world to treat bacterial infections. However, penicillin is not a novel discovery synthesized in a lab. Penicillin is produced by *Penicillium* fungal mold.[27] The agent was first discovered when the fungus grew on an agar plate with *S. aureus* and inhibition of growth was observed. Penicillin's mechanism of action is by inhibiting cells from constructing a functional cell wall by binding to proteins required for crosslinking. Without a proper cell wall, bacterial cells are unable to control the entry and exit of the compounds and therefore cannot create a homeostatic intracellular environment necessary to complete biochemical functions. Natural penicillin is still used as a medicine today, but has a limited spectrum of antibacterial activity due to resistance. Synthetic analogues of penicillin have been synthesized in order to increase potency and target a larger array of pathogens.[27]

In order to make synthetic modifications, the structure of penicillin was first explored. All penicillin derivatives share three common structural features: a thiazolidine ring, an attached β-lactam ring, and a side chain.[27]

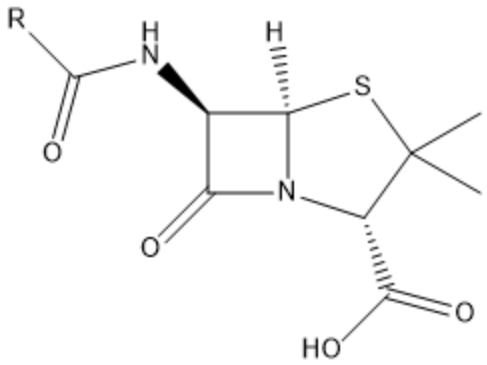


Figure 6: The general structure of penicillin is shown above. The central 4 ring cyclic group is a β-lactam ring, which can be hydrolyzed by β-lactamase in resistant strains. The 5 membered ring with the sulfur attached to the β-lactam ring is a thiazolidine ring. The R group can be modified synthetically in order to increase the diversity of penicillin drugs.[28]

Like all antibiotic agents, resistance has become an increasingly large issue. Resistant strains of *S. aureus* produce an enzyme known as β-lactamase which is able to hydrolyze the β-lactam ring in the structure of penicillin.[28] Encountering resistance encourages the development of synthetic agents by modifying existing drugs to counter resistant mutations. Additionally, searching for novel targets that do not have any known developed resistance is an alternative to treat resistant infections.

Chapter 4: Necessary Drug Properties:

Drugs must contain the necessary potency as well as pharmacokinetic properties allowing them to be administered safely within the human body, not simply only be effective in an *in vitro*

environment. These properties generally include absorption, distribution, metabolism, and excretion.[29]

Absorption refers to the rate that the drug enters the bloodstream. Multiple different factors can influence the rate such as how it is administered, interactions with food, and the chemical properties of the drug, polarity and lipophilicity. Drugs that act immediately are generally administered through an injection directly into the bloodstream, however more commonly used drugs are administered orally through pills. Generally it is more desirable to administer pills orally for convenience, so factors such as these are taken into consideration when optimizing a drug. Oral pills enter the bloodstream through digestion generally in the small intestine. These drugs also end up being absorbed in the liver and pancreas where enzymes metabolize compounds in the body. When designing a pill, factors such as how the drug is metabolized during digestion are taken into account as it will likely not be 100% bioactive post digestion. When administered intravenously, the compound enters the bloodstream directly and prevents this issue.[29]

Distribution of a drug refers to how the drug travels within the body. How the drug travels in the body determines how much of the drug is distributed in different tissues such as the brain, blood stream, and liver. This creates important toxicity and potency questions as drugs may travel to destinations that lead to toxicity and may not arrive in the intended active site due to different properties based on molecular structure.[29] In general, drugs are not designed to travel to any certain location, but are evaluated in animal models.To determine the distribution of drugs in animal models, the compound is radiolabelled then traced to different tissues.[30] If a drug does not end up in its proper target location, then it must be redesigned.

Metabolism generally refers to how the body breaks down compounds into molecules that can be excreted. Enzymes known as cytochrome P450s (CYP 450) are responsible for the metabolism of a large majority of pharmaceuticals on the market.[30] Metabolism is important in the drug discovery process as factors such as the speed of which a drug is metabolized will affect efficacy. There is also a concern about drug-drug interactions. For patients that are taking more than one drug, the metabolism by CYP450s can be affected. This can lead to changes in the pharmacokinetics of drugs leading to longer or shorter exposure to the drug.[30]

Lastly, excretion is the final major factor of concern. It is important to be aware of how compounds are excreted so they can be eliminated safely.[30] Antibacterials can be excreted in the feces or urine. It is important to develop compounds that are able to safely pass through the kidneys without causing any biological harm. It is well known that aminoglycoside antibacterials can cause nephrotoxicity. Because of this, it is extremely important to dose drugs at the lowest effective dose possible since most drugs have side effects. Many biologically active compounds interact with more than one specific protein, so balancing desired effect and side effects needs to be carefully considered in the development of any compound. Judgment needs to be applied in deciding if developing a potentially toxic drug may cause more harm than good at any given dosage. If any pharmacokinetic properties are not met, the compound must be redesigned before it is able to become a drug.[30]

An example of pharmacokinetics in action is how the anticoagulant drug, warfarin, works in the body (Figure 7). While warfarin is not an antibiotic, it is a perfect example of how all the pharmacokinetic properties play out in the drug design process. The same principles are applied to all antibiotic compounds that are prescribed. All four principles of absorption, distribution, metabolism, and excretion were carefully balanced to provide effective dosage and therapy to

reduce blood pressure and decrease the risk of heart attacks and strokes. Warfarin is prescribed to patients that are at risk for blood clotting.However inappropriate dosings can lead to the inability of blood to clot/coagulate,which has serious health and toxicity risks.[31]

Warfarin is administered through an oral dosage that absorbs over time within the body. Studies have shown that 100% of the drug is absorbed and the concentration of the drug peaks between 3 and 9 hours after being ingested.[31] As this type of drug is not essential to act immediately when administered to prevent long term blood clotting, the slow absorption is not a limiting factor. The kinetics of a drug such as a fast-acting pain reliever require a different absorption rate. It is also important in the case of warfarin to understand that the concentrations do not peak for several hours after administration. Drug concentrations in the blood vary over time. Therefore, understanding the time course of absorption is important in order to prevent overdosing.[31]

Distribution of warfarin leads to relatively high levels in the bloodstream. The drug eventually concentrates in the liver which is where the drug becomes active in the body. As the drug is absorbed by the liver, the molecule works by inhibiting the production of vitamin K which is involved directly in the ability for blood to clot. The distribution of the drug relates directly to the slow absorbance since it takes time for the drug to reach the liver where it is bioactive and produces the anticoagulant effect. The drug is then metabolized slowly in the liver.[31]

Since warfarin is given as a racemic mixture of S and R enantiomers, different enzymes are used to metabolize the drug. The R configuration is metabolized by CYP1A2 which produces 6- and 8- hydroxywarfarin while CYP3A4 produces 10-hydroxywarfarin. These metabolites are then further broken down by carbonyl reductases into alcohols. The S configuration is

metabolized by CYP2C9 into 7-hydroxywarfarin. The drug is then able to be excreted by the body through urine. The ability for the body to break down drugs is important in preventing accumulation of these potentially lethal substances. Our bodies are equipped with these CYP enzymes for metabolism and excretion. These parameters must be optimized when developing a drug. Number some of the positions on the structures for clarity.[31]

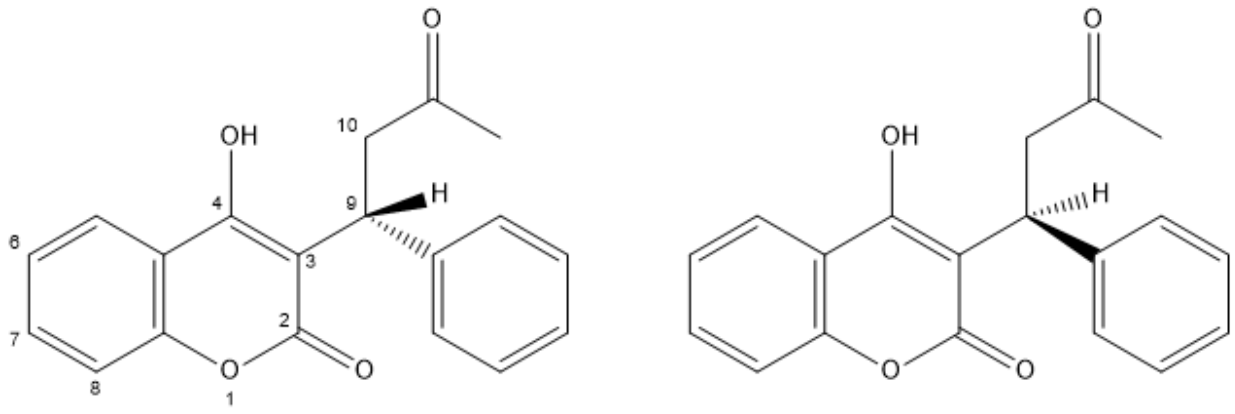


Figure 7: 2D structure of warfarin is shown above. Chirality of carbon 4 determines the R (left) or S (right) warfarin present. Since the drug is given as a racemic mixture, both molecules are present and are broken down slightly differently by the body, however the effects of the drug remain the same.[31]

Successful *in vitro* studies in the lab are not sufficient to develop a drug. How effective the drug is *in vivo* is critical. As shown, many factors are at play when developing pharmaceuticals before they can be prescribed for patients. When designing a drug, all of these factors demonstrated by warfarin must be fully examined and understood before a drug can be approved by the FDA, regardless of how effective they are *in vitro*.

Chapter 5: Isoindoles

Isoindole compounds have been discovered for over a century with many known biologically reactive properties. The isoindole backbone structure is used in this research to develop novel antibiotic compounds. An isoindole is composed of a fused benzopyrrole ring system that constitutes the regioisomer of the abundant 1H-indole heterocycle.[32] Since these molecules are the base of many pharmaceutically active compounds, a general synthesis is useful for the production of the backbone structure. A general Diels-Alder reaction can be used to conjugate the pi-system in order to produce the structure.[32]

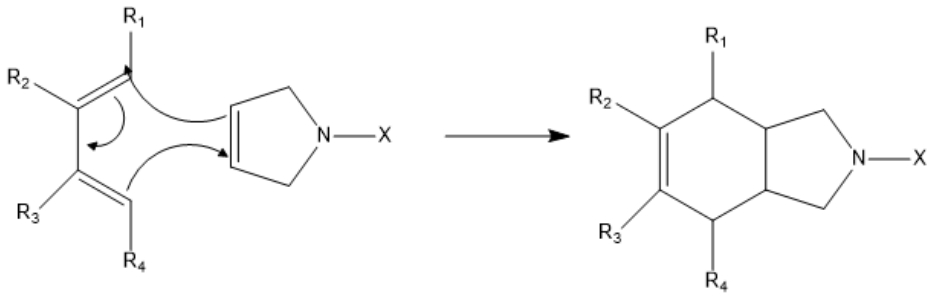


Figure 8: The general diels alder mechanism to synthesize the backbone of the isoindole structure. Flexibility of R groups allows for diversification of backbone before undergoing any further synthesis.[32]

Naturally occurring isoindoles are also found from various natural sources. The antimicrobial isoindole, 6-methoxy-2,5-dimethyl-2H-isoindole-4,7-dione (Figure 9) was discovered from the *Renira* sponge. Multiple reaction schemes were generated in order to synthesize this compound and learn more about the biological activity. However, the mechanism

of inhibition is not clear and the compound has not progressed into a pharmaceutical compound at this time.[32]

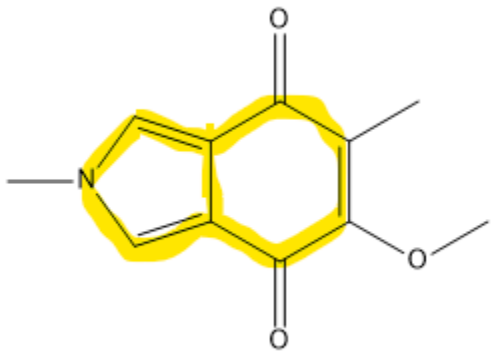


Figure 9: Structure of naturally occurring 6-methoxy-2,5-dimethyl-2H-isoindole-4,7-dione with isoindole backbone highlighted in yellow.[32]

Staurosporine is an indolocarbazole, a complex class of compounds containing isoindoles, that was isolated from *Streptomyces staurosporeus* in 1977 (Figure 10).[32] Since this discovery, over 60 compounds that fall into this subclass of isoindoles have been discovered with 26 of these compounds displaying strong antimicrobial activity. Each of these compounds were found to inhibit protein kinase activity, which are important signaling enzymes. After studying the crystal structure of these kinases as well as the molecular structure of these compounds, it was found that they inhibit the kinases by binding in an adenosine pocket which leads to cytotoxic effects.[32]

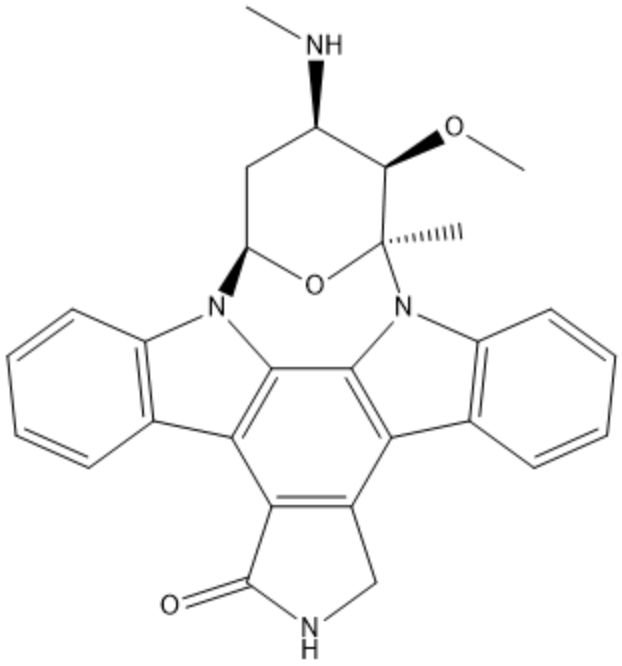


Figure 10: 2D structure of Staurosporine, a potent isoindole compound. Multiple isoindoles can be identified within the structure.[32]

Since protein kinase activity is displayed at a higher rate in cancer cells, research has gone into developing an anticancer drug therapy based on the structure of these indolocarbazole compounds.[32] An anticancer agent would need to have protein kinase inhibitory selectivity so that normal cell growth will not be inhibited resulting in cytotoxic effects. The drug Imatinib (Figure 11), which is now a currently marketed drug to treat leukemia, is the result of a specific kinase inhibitor.[33]

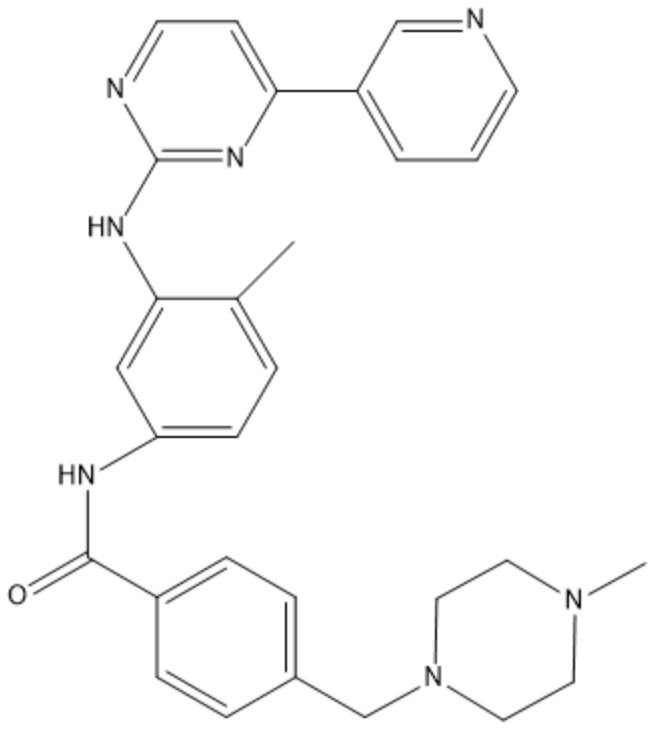


Figure 11: Structure for Imatinib, a marketed selective kinase inhibitor for leukemia cancer cells.[32]

Additional antibacterial properties of isoindoline, a class of compounds known as isoindoline-1,3-diones, have shown pharmacological promise.[34] This class of compounds has been shown to have multiple biological activities. These compounds not only have antimicrobial activity but have also shown anti-inflammatory, anthelmintic, insecticidal, anticonvulsant, antitubercular, and analgesic activity (Figure 12).These compounds are known to inhibit cyclooxygenase as well as acetylcholinesterase. Pyridine derivatives specifically were shown to have exceptionally high antibacterial activity and have been considered for pharmacological use. As of 2015, several of these derivatives were synthesized and tested *in vitro*. Pharmacokinetic

properties were determined for several compounds. However conclusive evidence did not lead any of these compounds advancing into clinical trials.[34]

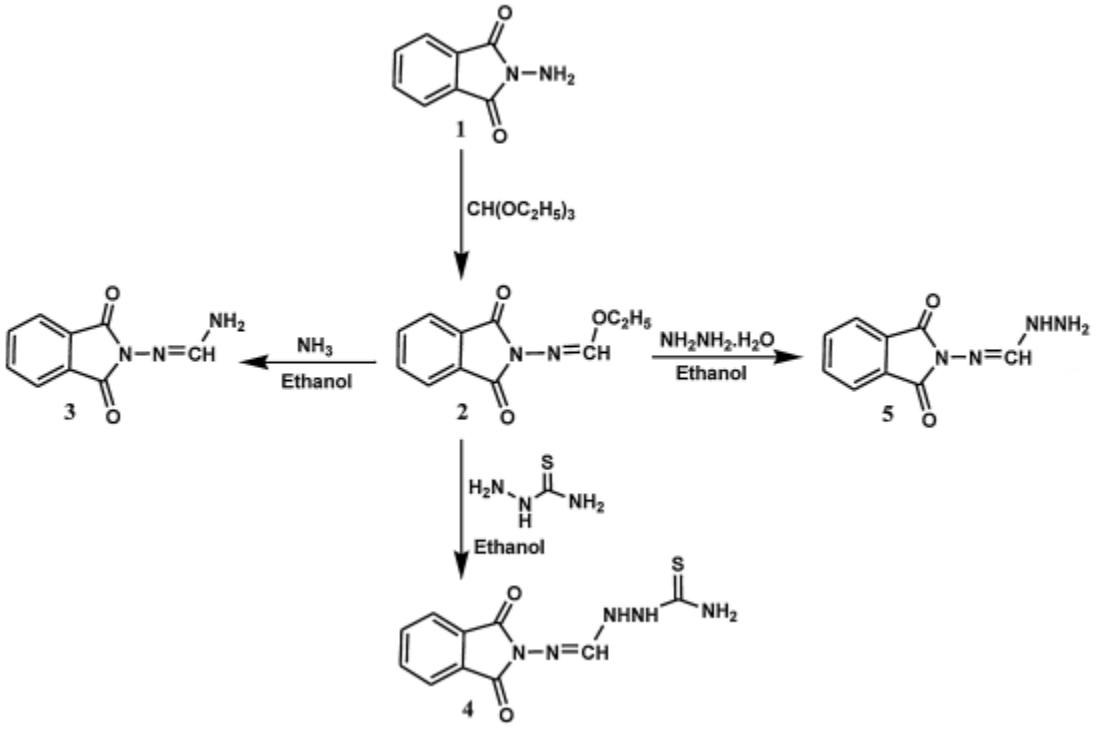


Figure 12: Isoindole 1-3 compounds that have shown antibacterial activity in *in vitro* assays. Rewritten with permission[35]

The compounds synthesized for this research utilize the isoindole backbone to inhibit the growth of *S. aureus*. Since these compounds are biologically active, it is important in future work to additionally test for activity in mammalian cells to ensure specificity of the potential drug.

Chapter 6: FTSz

Bacterial organisms replicate far differently than multicellular organisms. Binary fission is an essential process by which a bacterial cell is split into two daughter cells. A protein, FtsZ, is an important part of this process as it is the start of a signaling pathway that ultimately leads to cells being able to physically divide. Without FtsZ functioning properly, bacterial cells will reach the cytokinesis step in mitosis but fail to be able to divide thus leading to overall cell death.[35]

FtsZ is a protein that is found in both Gram-positive and Gram-negative bacteria.[35] Crystal structure analysis has shown FtsZ to be largely conserved across bacterial species, however not completely identical. This means that while a drug may be able to inhibit a specific FtsZ protein from *S. aureus*, it may not be able to inhibit an FtsZ protein from *E.coli*. A broad spectrum antibiotic that uses FtsZ as a mechanism of inhibition may be difficult to achieve. It is still worthwhile to develop FtsZ inhibitors, especially for bacteria that are already resistant to other widely used antibiotics such as methicillin resistant *S. aureus*. It was also found in this study that FtsZ structures within genera of bacteria were not significantly different, meaning that a compound that works on *Staphylococcus* would likely inhibit the growth of all species within that genus.[35]

During cytokinesis, FtsZ is believed to be the first protein to migrate to the center of where the cell will divide to form the start of what is known as the Z ring.[36] From here, FTsZ recruits several other proteins shown in Figure 13 such as ZipA, FTsQ, FTsA, and FtsW to the center of the cell. Once these proteins are all recruited to the center of the cell, cytokinesis takes place and the cell divides into two identical daughter cells.

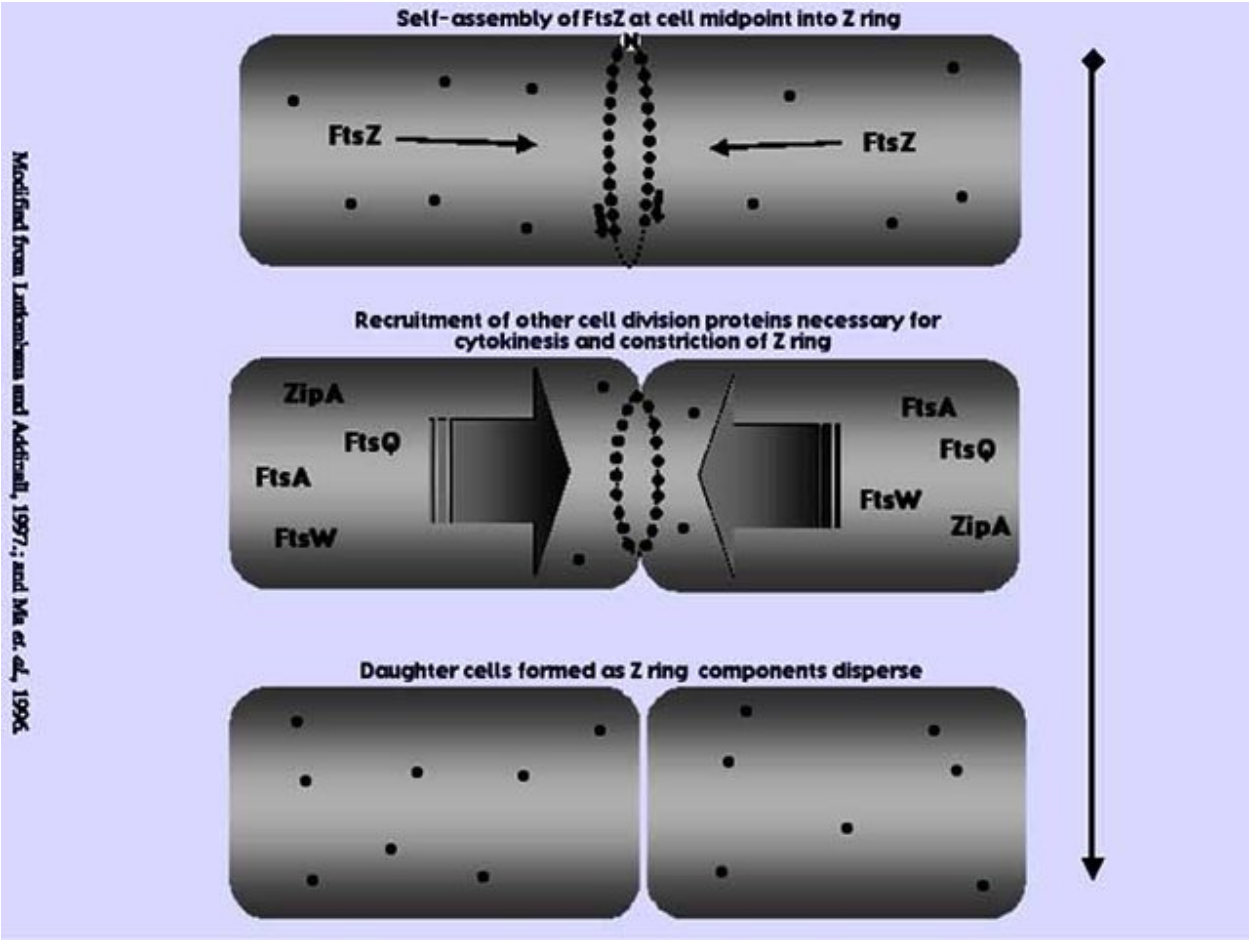


Figure 13: Overview of the formation of the Z ring by FtsZ protein as well as recruitment of other essential proteins for cytokinesis. Rewritten with permission[37]

FtsZ is an analogue of the eukaryotic protein tubulin and both proteins are polymerized by GTP.[38] While there is an analogue known as tubulin in human cells, studies show that molecules that inhibit FtsZ will most likely not inhibit the tubulin proteins based on structural differences between tubulin and FtsZ.39 This makes FtsZ a good target since that means that it will likely have less toxicity to human cells since tubulin would not be affected. By inhibiting FtsZ selectively, the cytokinesis pathway in bacteria will be disrupted and additional proteins will not be

recruited to allow the cell to complete the mitosis process.[38]

FtsZ has not been successfully targeted by any pharmaceutical company although many attempts have been made. As of 2020, the most advanced compound that targeted FtsZ,TXA709, failed in stage 1 clinical trials due to poor pharmacokinetics.[38]. Pharmaceutical companies will continue to pursue FtsZ inhibitors since the protein presents a novel essential target with no known natural resistance.

Understanding the structure of FtsZ can allow us a better understanding of how to possibly inhibit its function. FtsZ is composed of 23 residues divided into two domains, the N-terminus and C-terminus linked by an alpha helix (Figure 14). The active site for GTP binding is located between two FtsZ monomers at the H7 helix .

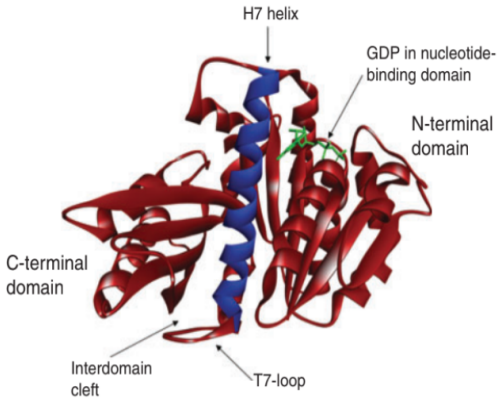


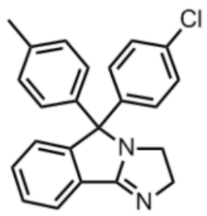
Figure 14: Crystal structure of FtsZ protein adopted with permission from Bottomley 2019. Computer modeling predicts that the compound synthesized in the lab binds to the interdomain cleft shown at the bottom left of the structure.[39.]

Three druggable targets have been identified within the protein structure to aid researchers in the development of new antibiotic compounds.[35] These regions are the nucleotide

binding domain (NBD), the T7-loop, and the interdomain cleft. When forming the Z-ring, FtsZ inserts the T7-loop into the nucleotide binding domain of another FtsZ protein to form a closed structure. In an *in vitro* study it was found that a substituted C8 GTP analogue could selectively bind and inhibit the nucleotide binding domain of FtsZ while unaffecting tubulin protein. The T7-loop has also been identified as a target because of a study that found small molecules can bind into this pocket in NMR analysis. Also, the interdomain cleft located between the central core domain and the C-terminus has been successfully targeted by a compound, PC190723. A computational study showed this compound was able to bind with Val 207, Leu 209, and Asn 263 all located in this interdomain cleft.[40]

Chapter 7: Where we began

Organic synthesis of novel antibacterial compounds began by screening several hundred compounds prepared by a previous RISE Fellow, Dr. William Houlihan. Dr. Houlihan was synthesizing compounds for the treatment of cocaine addiction and to help in weight loss. A family of 13 compounds was discovered with similar structures that produced a zone of inhibition against *S. aureus* when screened with an agar diffusion assay (Figure 15). The core structure contained an imidazoline. In order to improve the antibacterial potency, a five step synthetic route was developed and employed by Drew students to generate analogues. (Figure 16).



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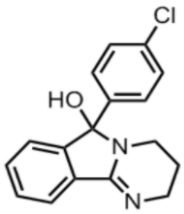

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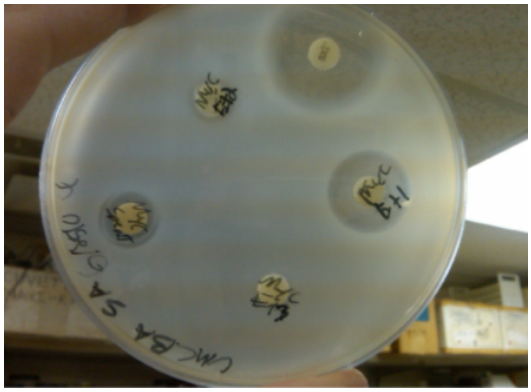


Figure 15: On the left are the initial imidazoline structures discovered from compounds synthesized by RISE fellow Dr. Houlihan. On the right is the *S. aureus* agar diffusion assay.

Based on the results of this work, Dr. Gullo's students discovered the antibacterial biological activity of these compounds. It was recognized that the final imidazoline compounds had less antibacterial activity than the isoindole analogues. Based on this observation, the final cyclization step was excluded from the general synthesis. The project then focused on the synthesis of new isoindole compounds. (Figure 16)

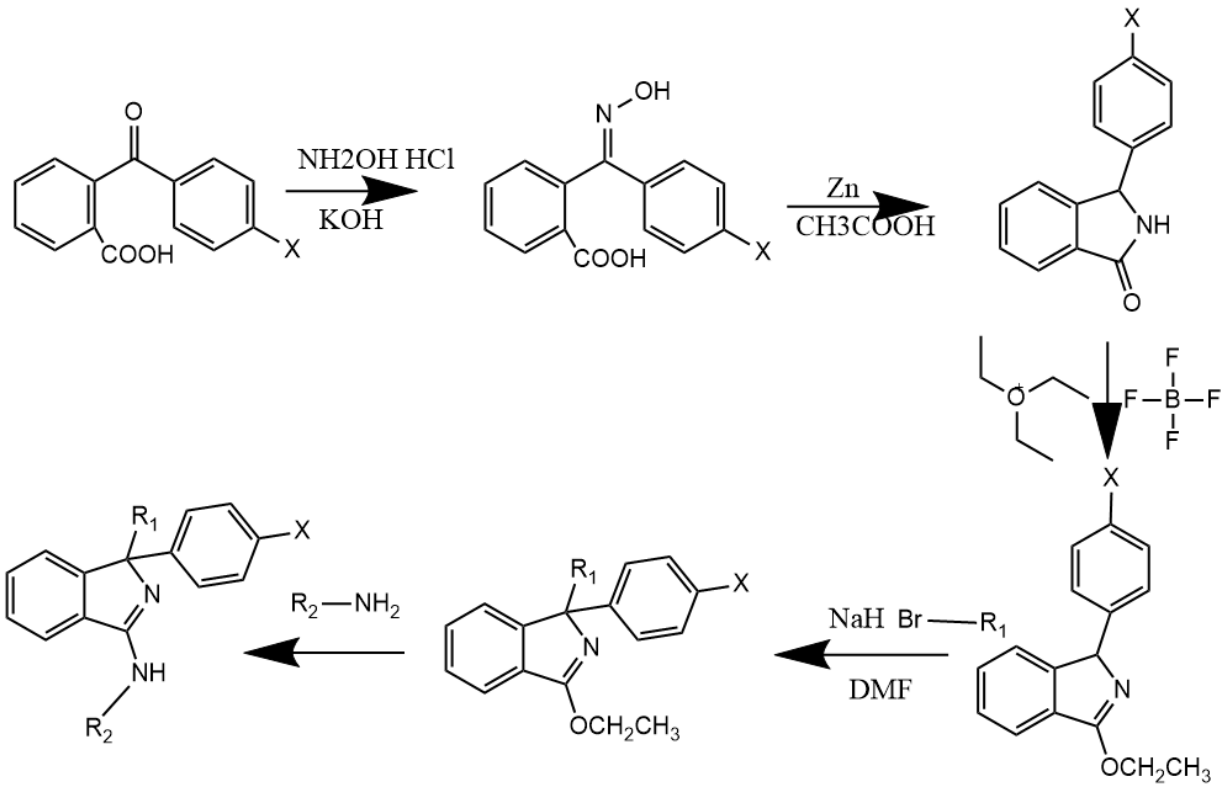


Figure 16: General synthesis from starting material to biologically active isoindole compound. Location X is a halogen while R1 and R2 groups can be modified.

Modifications of the isoindole structure were easily accessible at two locations using this synthetic scheme, (Figure 16). Drew students have synthesized analogs of the isoindole core structure to determine the structure activity relationship (SAR) for antibacterial activity..

A former student, Benjamin Strickland, evaluated the most potent compound in SEA, Similarity Ensemble Software, a database available from UCSF that indicated potential inhibition of FtsZ. When we analyze the structure of FtsZ in Figure 14, we are able to observe three n sites located on the structure where inhibitory compounds might bind, the interdomain cleft, the T-7 loop, and the GDP in nucleotide-binding domain.

Further work in CADD (Computer Assisted Drug Design) using Biosearch IT software supported this finding and predicted binding to the interdomain cleft of the FtsZ protein structure. In order to test the hypothesis that the target of the isoindoles is FisZ, an *in vitro* assay was performed in collaboration with the RISE Fellow, John Perkins. It is known that if FtsZ is inhibited by a sub-lethal dose of an FtsZ inhibitor that surviving cells would not be able to divide to complete the cytokinesis process and thus elongated cells would be observed. When a sub-lethal amount of compound was tested with *S. aureus* cells, elongated cells were observed, supporting the hypothesis that the compound inhibited bacterial growth by inhibiting FtsZ proteins.

The following research thesis is an organic synthesis project of novel isoindole antibacterial compounds with the objective to discover potent antibacterials. The ultimate goal is to synthesize a novel isoindole compound with antibacterial potency similar to marketed drugs with a new antibacterial mechanism of action. This research report will describe a 5 step synthesis and the antibacterial activity of these compounds. Once the final product has been synthesized, a bioassay was performed in order to assess the antibacterial potency of the compounds. Assessment of whether the compound is more or less potent than previously synthesized compounds will provide insight into designing future analogues.

Chapter 8: General Methods

Analytical LCMS was taken on a Waters LCMS with an Echelon-C18 4µm 100x4.6mm. The solvent system used was a gradient of 10% acetonitrile: 90%, 0.1% formic acid to 90% acetonitrile;10%, 0.1%formic acid with a flow rate of 1.5 mL per minute over 15 minutes.

Preparative HPLC: Preparative HPLC was performed with a Waters HPLC with an Echelon-C18 4µm 150x20mm column. The solvent system used was a gradient of 10% acetonitrile: 90%, 0.1% formic acid to 90% acetonitrile; 10%, 0.1%formic acid with a flow rate of 10 mL per minute over 30 minutes.

NMR was taken on a Bruker 400 MHz NMR. All samples were run in $CDCl_3$

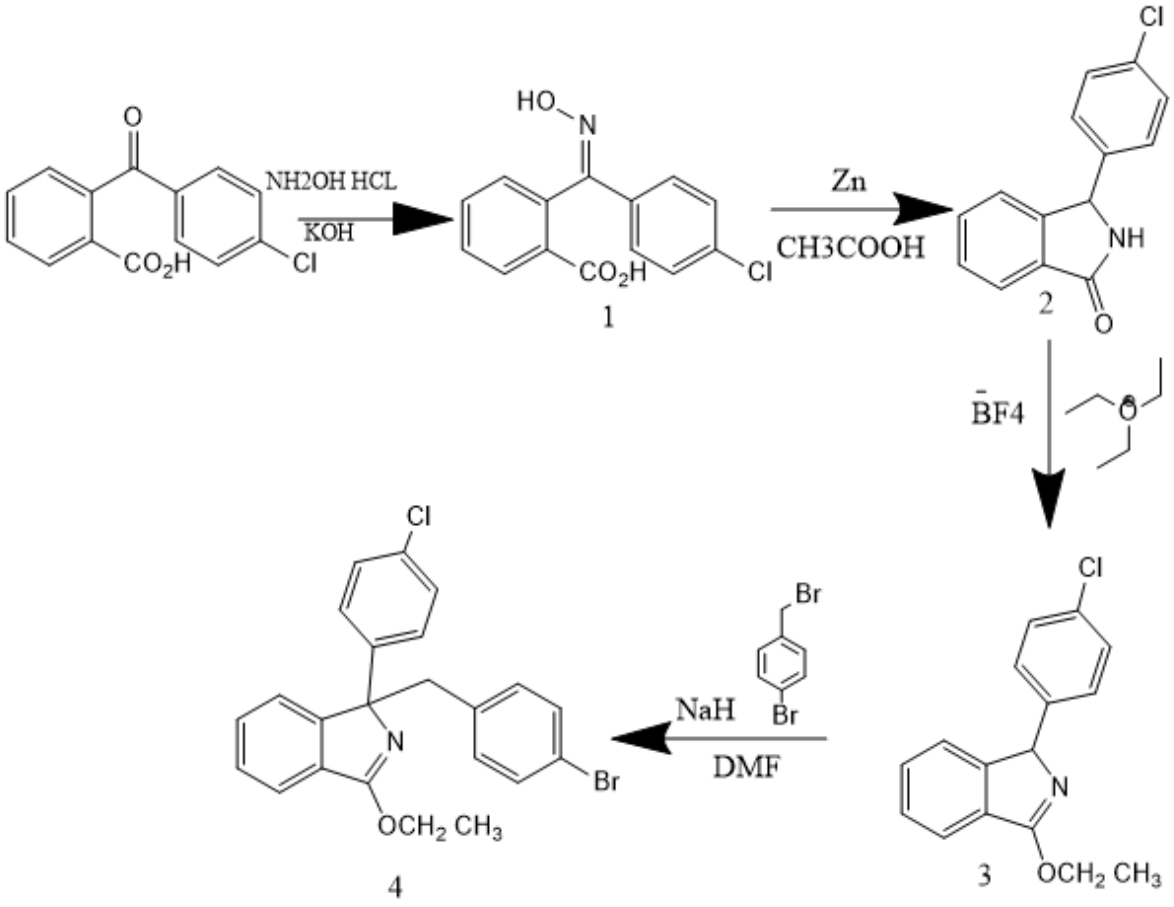
Silica gel was Fischer silica gel grade 100 35-70 mesh The solvent system employed 95% hexane:5% ethyl acetate or 97% hexane:3%ethyl acetate.

Bioassay

An agar diffusion assay was performed on Mueller Hinton Agar plates. Phosphate buffer, 20mL of 1X phosphate buffer, was prepared from a 10X sterile stock solution. *S. aureus* (20µL) obtained from the microbiology lab freezer stored at -80℃ was added to the 1X phosphate buffer. Buffer mixture (3mL) was spread evenly on each agar plate, the excess removed and then left to dry in a sterile hood. Six, two fold dilutions of the test compound, were made with a starting concentration of 4 mg per L in methylene chloride. Each 6mm sterile paper disc was then pipetted with 20µL of solution resulting in the highest concentration disc containing 80 µg of compound. Discs were placed on agar diffusion plates once fully dried. The control used in the experiment was 10 µg of Ciprofloxacin. Plates

were incubated overnight at 37℃. The zone of inhibition was measured by taking the diameter of each halo across the disc.

Chapter 9: Synthesis of synthetic intermediate 4



Scheme 1: Overall 4 step synthesis of synthetic intermediate 4.

Reaction 1

KOH, 42.43g (0.756 moles) and 19.45g (0.0746 moles) of 2-4-chlorobenzoyl benzoic acid were added to a flask containing 200mL of water. NH_2OH:HCl, 12.35g (0.178 moles), was dissolved in 40mL of H_20 and then added to the reaction flask. The reaction was refluxed for 30 minutes while being stirred. Once removed from heat, the flask was placed in an ice bath until cool.. Approximately 50mL of 12M HCL was added to the flask until the pH reached approximately 4. The reaction was then cooled for 10 minutes in an ice bath, and the resulting precipitate was isolated by vacuum filtration.

The product was transferred to a 2L flask with 1400mL of ethanol and heated to boiling. Once the contents fully dissolved, the flask was removed from heat and placed in a refrigerator to cool. The crystals were then isolated through vacuum filtration and were labeled 1 (yield 72.7%, 14.97g, 0.0543 moles obtained). Crystals were analyzed by LCMS and NMR, proton and carbon, (Figure 17) UV retention time, 4.41 minutes, MH+ 258.8, 260.8, H1 NMR 7.5-8.5 ppm [aromatic protons]; C13 NMR 122-136 ppm 12 [aromatic carbons], 156 ppm (imine carbon); 163 ppm (carboxylic acid carbon)

Reaction 2

Acetic acid, 350mL, and 14.97g (0.0534 moles) of compound 1 were added to a two neck flask and refluxed. Zn powder, 19.00g (0.29 moles) was added to the reaction flask and was left to continue to reflux for 30 minutes. Solution was then

removed from heat and left to cool. Once cooled, Zn solid was removed through vacuum filtration. The solution was then chilled in an ice bath until precipitate formed, which was then isolated through filtration. Once dried, the precipitate was added to a flask with 250mL of EtOH and heated to boiling . Water, 200mL, was added slowly in 50mL segments to produce a cloudy solution. The solution was then left to cool in the refrigerator. Once cooled, vacuum filtration was used to recover crystals that were then labeled compound 2 (62.7%, 8.87g, 0.0365 moles recovered). Crystals were analyzed by LCMS and NMR, proton and carbon.. (Figure 18) UV retention time, 8.1 minutes, MH+ 243.5, 245.3.7, H1 NMR singlet 5.6ppm , 6.7-7.9 ppm [aromatic hydrogens]; C13 NMR 60 ppm [non aromatic carbon] 171 ppm [carbonyl carbon] 123-148 ppm [aromatic carbons]

Reaction 3

Compound 2, 5.91g (0.0243 moles), was dissolved in 60mL of methylene chloride. Triethyloxonium tetrafluoroborate, 9.60g (0.0505 moles) was added to the reaction flask and left to stir overnight. An LCMS was taken which indicated the product had formed. The reaction mixture was then washed twice with 60mL of sodium bicarbonate using a separatory funnel. $MgSO_4$ was then used to dry the methylene chloride solution. Methylene chloride was then removed using a rotary evaporator to isolate crude reaction product solid.. The compound was then chromatographed on a silica gel column using 95% hexane:5% ethyl acetate as the elution solvent system. Fractions containing the product were concentrated on the rotary evaporator and the resulting compound was labeled compound 3 (49.7%,

3.26g 0.0120 moles recovered) and both a carbon and proton NMR were obtained. (Figures 19) UV retention time, 9.1 minutes, MH+ 272.8 ,274.8, H1 NMR triplet, 1.4ppm [CH3], quartet, 4.5 ppm [CH2], singlet 5.6 ppm [CH] 7.0-7.7 ppm [aromatic protons]; C13 NMR 14.5 ppm [CH3}, 64.2 ppm [CH], 71.7 ppm [CH2], 121-153 ppm [aromatic carbons] 170 ppm [imine ether carbon]

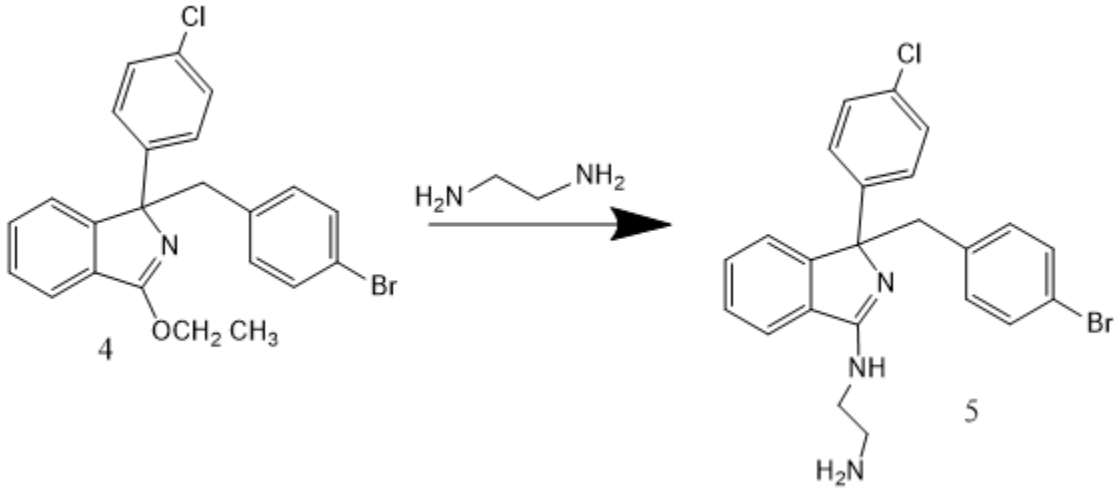
Reaction 4

Compound 3 (2.92g 0.0108 moles) and NaH (0.936g 0.0390 moles) were added to a sealed flask and placed under nitrogen in an ice bath. 45.6mL of dimethylformamide(DMF) was added and the reaction was left to stir for 1 hour. In a separate flask, 5.06g of para- bromo- benzyl bromide was solubilized in 19mL of DMF and added to reaction under nitrogen. After another hour, 40ml of water was added and the reaction mixture was transferred to a separatory funnel. The compound was extracted 2 times with 90 ml of diethyl ether (Et_2O). The ether solution was then washed with 30mL of NaCl(s) water and then with 30mL of water. The ether solution was dried with $MgSO_4$ then vacuum filtered. The Et_2O was then removed with a rotary evaporator.The precipitate was then chromatographed on a silica gel column with 97% hexane:3% ethyl acetate as the eluting solvent. Fractions containing the product were combined and the solvent was removed on the rotary evaporator . The resulting solid was labeled compound 4 (34.5%, 1.6g, 0.00364 moles obtained). An analytical LCMS was taken as well as both proton and carbon NMRs were obtained. (Figures 20) UV retention time 13.0 minutes, MH+ 439 441 443, H1 NMR triplet 1.5 ppm [CH3] singlet 3.5 ppm [CH2] quartet 4.5 ppm [CH2] 6.8-7,8 ppm [aromatic protons] C13 NMR 14.8 ppm

[CH3], 45.1 ppm [benzyl carbon] 64.1 ppm [C], 71.4 ppm [CH2], 121-143 ppm [aromatic carbons] 168.2 ppm [imine ether carbon].

Chapter 10: One step Synthetic Products

DCB 1-14-2 Ethyl Compound:

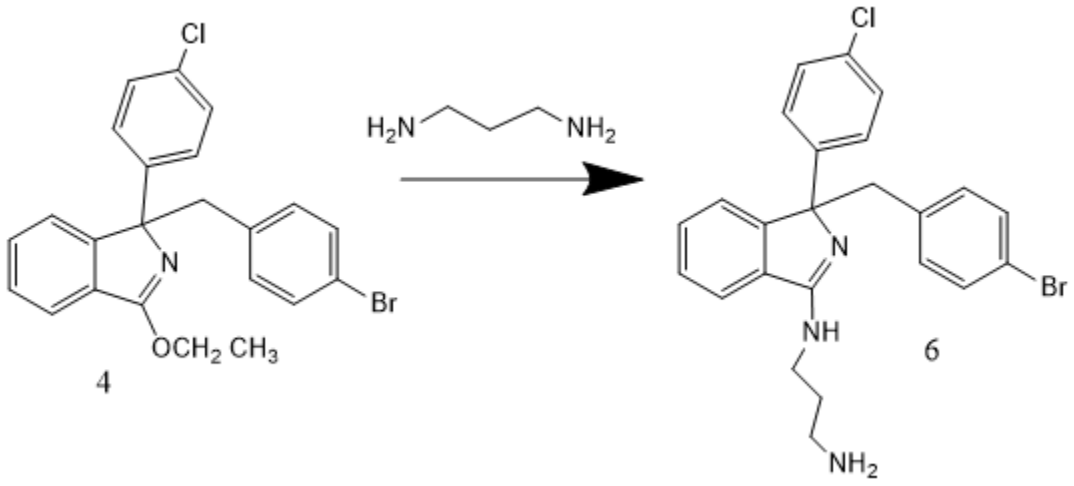


Scheme 2: One step synthesis of ethyl amine compound from synthetic intermediate 4.

Compound 4 (0.2g 4 *10^{-4} moles) and 5mL of 1,3-diaminoethane were added to a microwave tube. The reaction tube was then microwaved at 200℃ for 90 minutes. The reaction was evaluated by LCMS and the product was observed. A preparatory HPLC was run with a gradient from 10% acetonitrile:90% formic acid to 90% acetonitrile:10% formic acid. The fractions collected were evaluated by analytical LCMS and pure samples were combined. After evaporation of the pure fractions a solid was obtained (13%, 0.026g 5.74 * 10^{-6} recovered) and an

analytical LCMS was taken (Figure 21) UV retention time 5.52 minutes, MH^+ 454, 456, 458. An agar diffusion assay was then run using the bioassay procedure.

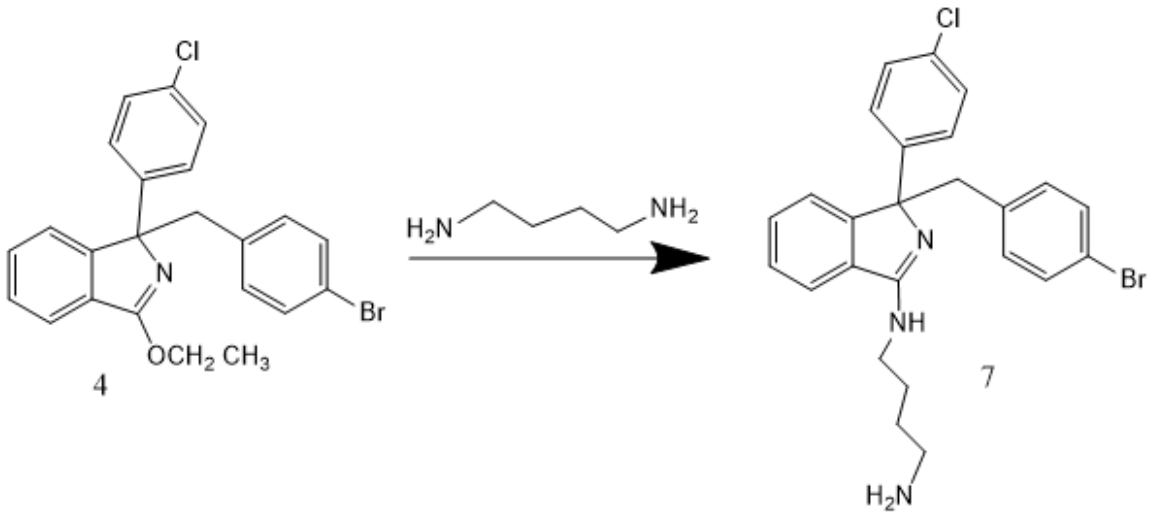
DCB 1-15-1 Propyl compound synthesis:



Scheme 3: One step synthesis of propyl amine compound from Compound 4.

Compound 4 (0.2g, 4 *10^{-4} moles) and 5mL of 1,3-diaminopropane were added to a microwave tube. The reaction tube was then microwaved at 200℃ for 90 minutes. The reaction was evaluated by LCMS and the product was observed. A preparatory HPLC was run with a gradient from 10% acetonitrile:90% formic acid to 90%:10% acetonitrile. The fractions collected were evaluated by analytical LCMS and pure samples were combined. After evaporation of the pure fractions a solid was obtained (67.8%, 0.128g, 2.74*10^{-4} moles recovered) and an analytical LCMS was run (Figure 22) UV retention time 5.5 minutes, MH^+ 468, 470, 472. An agar diffusion assay was then run using the bioassay procedure.

Synthesis of butylamine compound DCB 1-24-1:



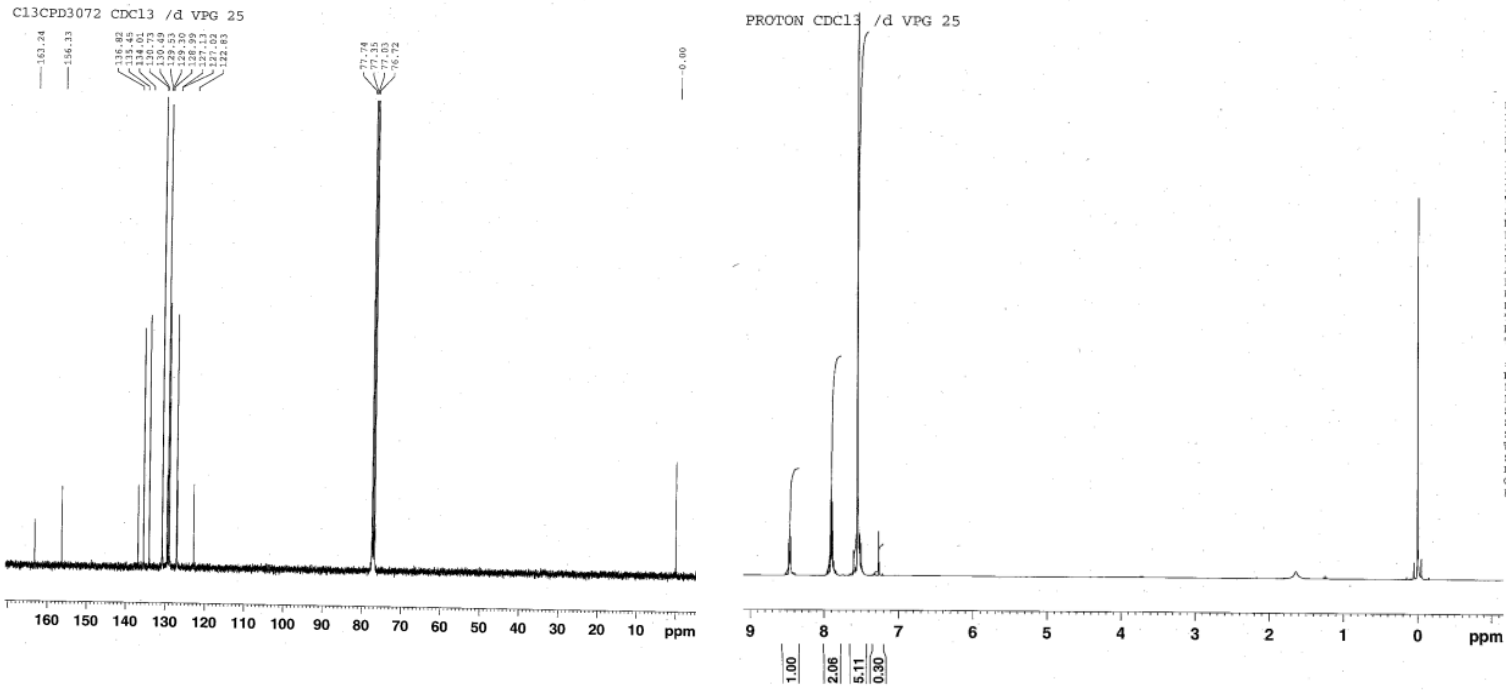
Scheme 4: One step synthesis of butyl amine compound from synthetic intermediate 4.

Compound 4 (0.2g 4 *10^{-4} moles) and 5mL of 1,3-diaminobutane were added to a microwave tube. The reaction tube was then microwaved at 200℃ for 90 minutes. The reaction was evaluated by LCMS and the product was observed. A preparatory HPLC was run with a gradient from 10% acetonitrile:90% formic acid to 90% acetonitrile:10% formic acid. The fractions collected were evaluated by analytical LCMS and pure samples were combined. After evaporation of the pure fractions a solid was obtained (29%, 0.058g 1.21 *10^{-4} moles recovered) and labeled compound 7. Product was confirmed by analytical LCMS (Figure 23) UV retention time 5.6 minutes, MH^+ 482 484 486. A bioassay was then run using the previous procedure.

Chapter 11: Results

Compound 1 was analyzed by LCMS as well as both proton and carbon NMR before moving forward (Figure 17). LCMS data confirmed the structure with a strong peak with molecular weight of 258 and 260. The presence of isotopes of chlorine was responsible for the observed peaks in the mass spectrum. Additionally, when we look simply at the total ion current the sample appears impure, however when paired with the UV spectrum we see a near 100% pure sample of compound 1 present. The total ion current is not a good quantitative measurement since different compounds ionize at varying degrees. We also notice that the molecular weight is MH^+ due to the fact that the analytical instrument ionizes the sample by adding a proton in order for the detector to get a reading. The UV spectrum allows for a more accurate analysis of the quantity of product in the sample compared to only looking at the ionization spectrum which shows all of the ions coming through the detector. Carbon NMR shows 12 peaks which accounts for all 14 carbons with 2 symmetrical pairs on the chlorinated aromatic ring. Proton NMR shows only aromatic protons present in the structure providing evidence of little to no impurities present..

A.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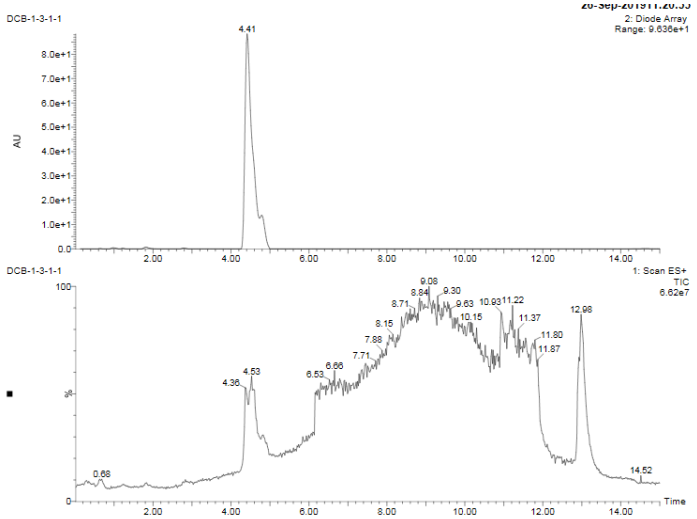
B.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C

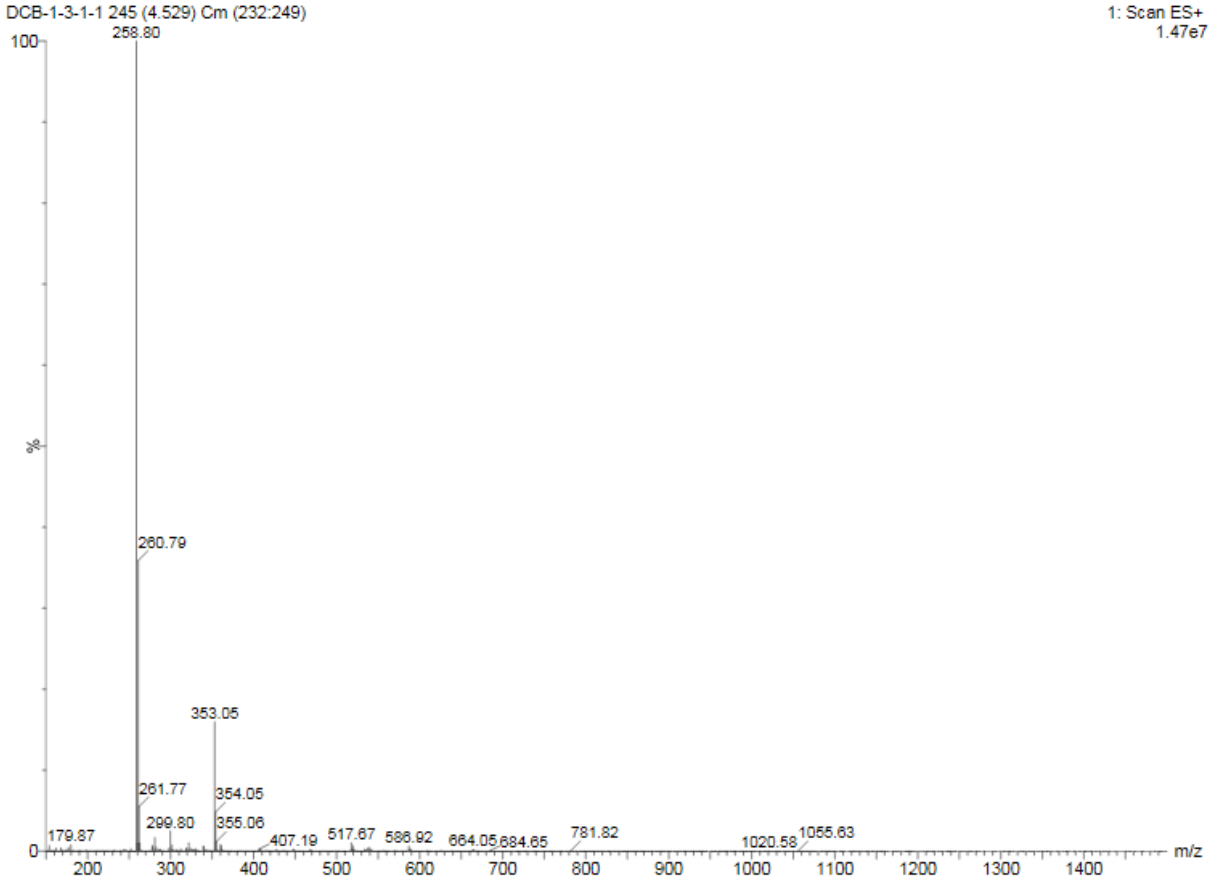
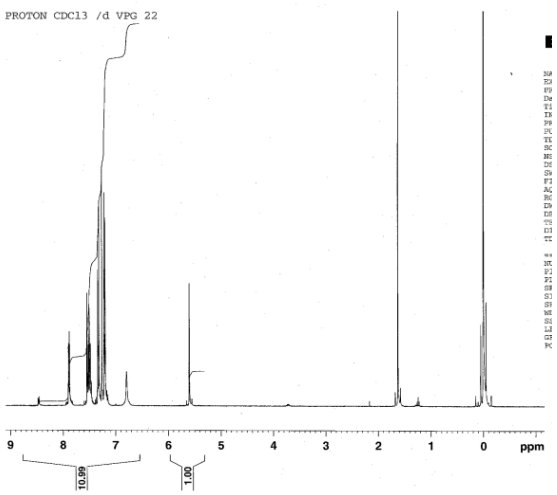


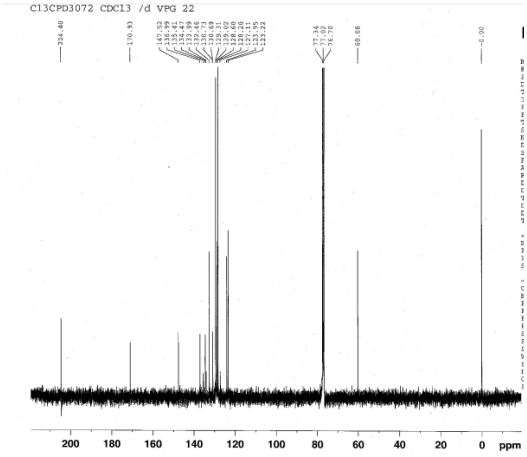
Figure 17: (A) Both proton NMR and Carbon NMR of 1 in $CDCL_3$. (B) Mass spectroscopy (bottom) and UV spectra (top) taken from analytical LCMS. (C) molecular weight taken from peak from (B) mass spectroscopy.

Compound 2 was also analyzed similarly (Figure 18). LCMS was used to confirm structure with strong peaks at 244, 246.In addition to the LCMS, proton and carbon NMR also helped identify the structure we predicted was present. The lone peak shifted to roughly 5.6 ppm could easily be identified as the lone proton attached to the carbon alpha to the nitrogen. When

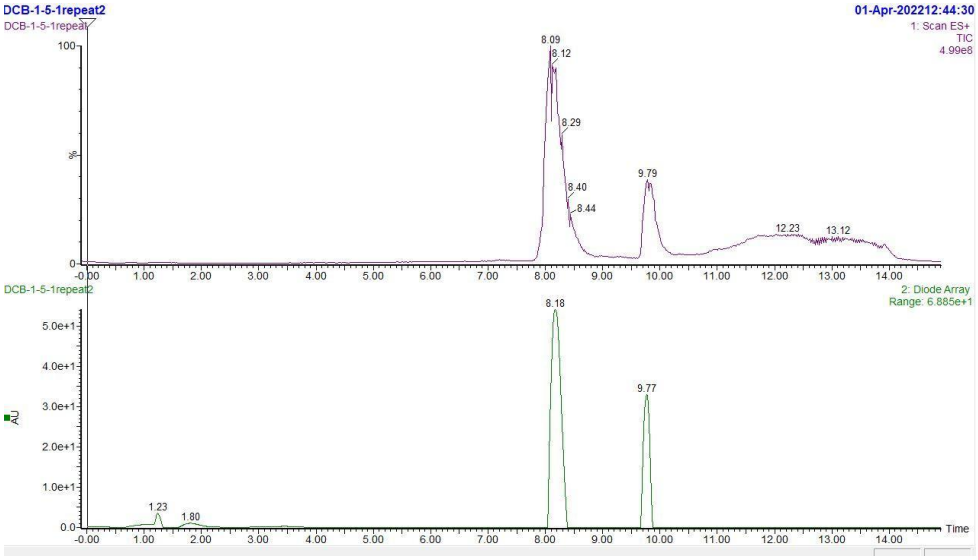
giving this peak an assigned integration of 1.0, the aromatic region integrated to 10.99 which accounts for the 8 predicted aromatic hydrogens as well as chloroform in the $CDCl_3$.. The carbon NMR also aligns with our predicted structure as we account for all 10 non-symmetrical aromatic carbons in our molecule. We can identify our single carbon alpha to the nitrogen located at roughly 60 ppm. We can also identify our amide carbon as a single peak shifted downfield to 171 ppm.

.A.





B.



C,

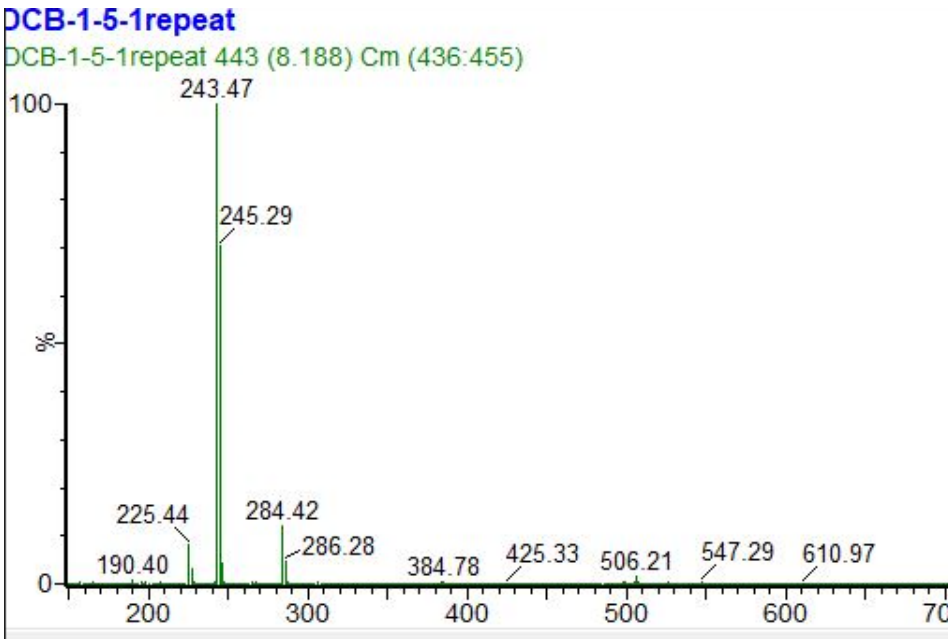
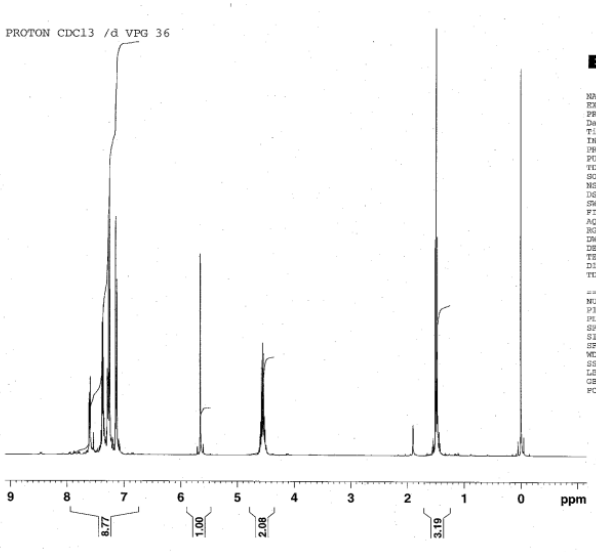


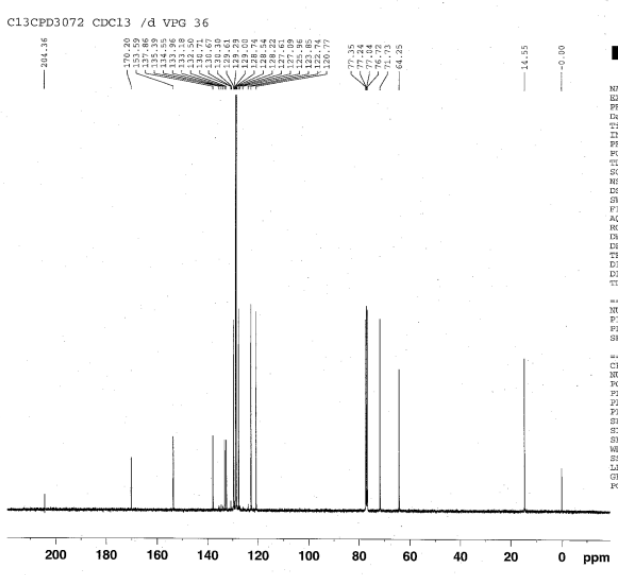
Figure 18: (A) Both proton NMR and Carbon NMR of 2 in $CDCl_3$. (B) mass spectroscopy (bottom) and UV spectra (top) taken from analytical LCMS. (C)

molecular weight taken from UV peak (B) from mass spectroscopy.

Continuing forward with our reaction sequence, compound 3 was also confirmed with the same techniques (Figure 19). The LCMS provides a large peak centered around 272 amu which aligns with the predicted structure. (Figure 19B). The proton NMR taken shows our distinct isolated proton at around 5.6ppm. When assigned an integration of 1.0, the peak at 4.7ppm has an integral of 2.09 corresponding to the 2 protons adjacent to the oxygen on the ether group. We also can clearly see our terminal methyl group peak with an integration of 3.19 at a shift of approximately 1.5ppm. This lastly leaves the aromatic region with an integration of 8.77 corresponding to the 8 aromatic protons on our predicted structure leading to very strong evidence. Looking at the carbon NMR, we also notice the emergence of 2 distinct peaks compared to previous compound 2. These peaks are located at roughly 15ppm and 75ppm, corresponding to the CH_3CH_2 respectively to the structure. All three analytical methods predicted that the structure of compound 3 was present with very little impurities.

A.





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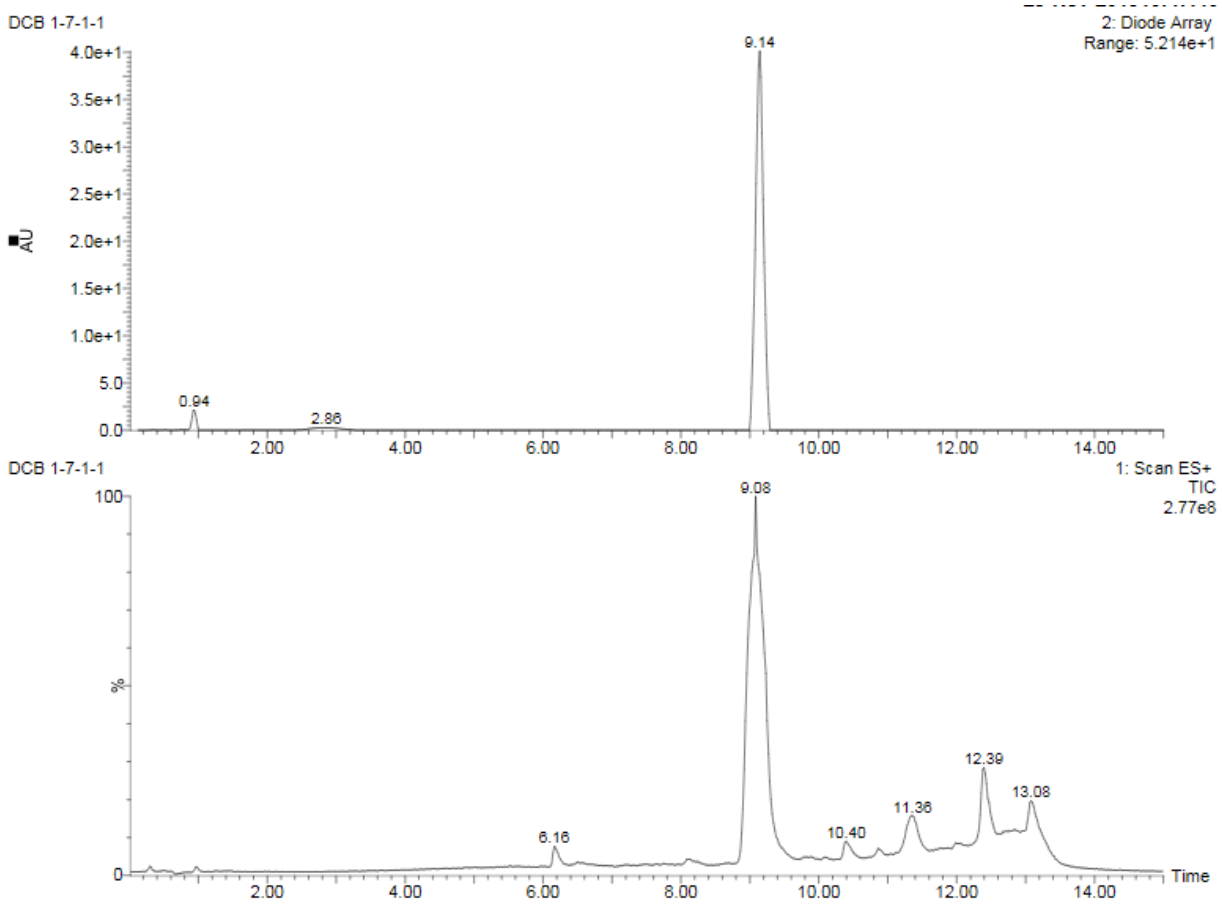
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1.0e+1
5.0
0.0
0.94
2.86
9.14
2.00
4.00
6.00
8.00
10.00
12.00
14.00
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10.40
11.36
12.39
13.08
Time

C.

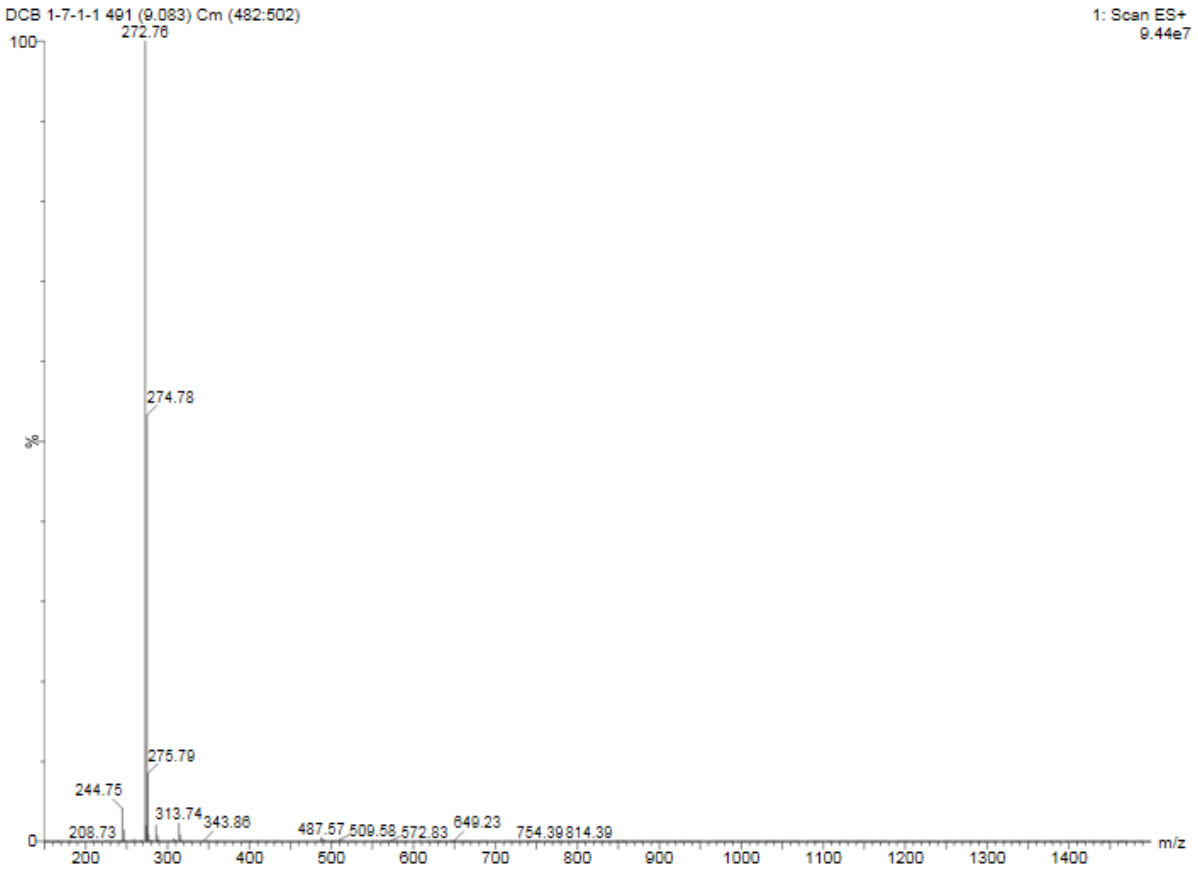
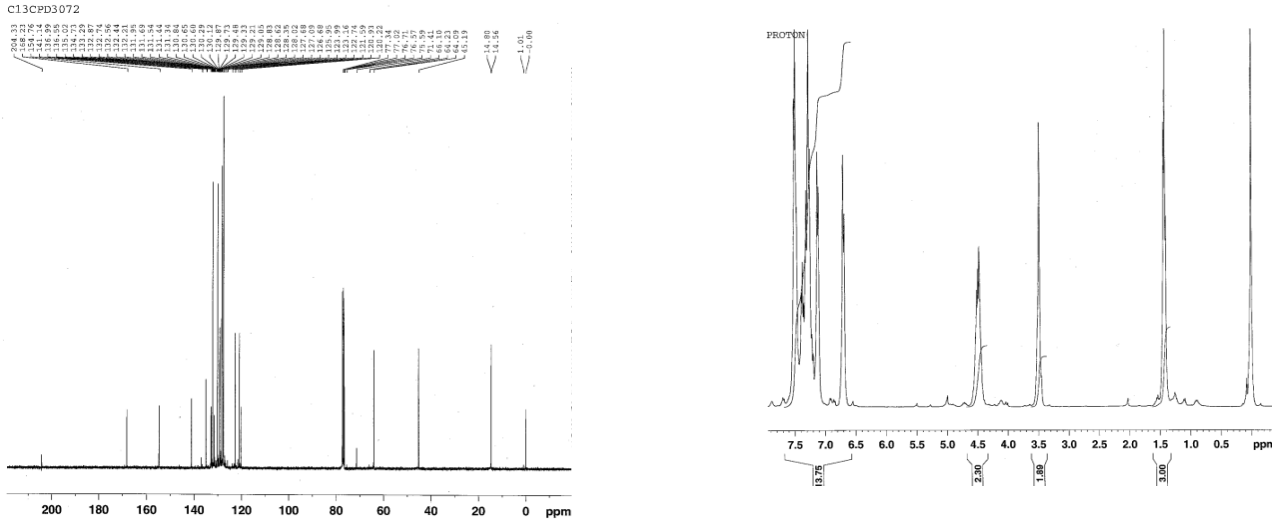


Figure 19: Both proton NMR and Carbon NMR of compound 3 in $CDCL_3$. B: mass spectroscopy (bottom) and UV spectra (top) taken from analytical LCMS. (C) molecular weight taken from peak from (B) mass spectroscopy.

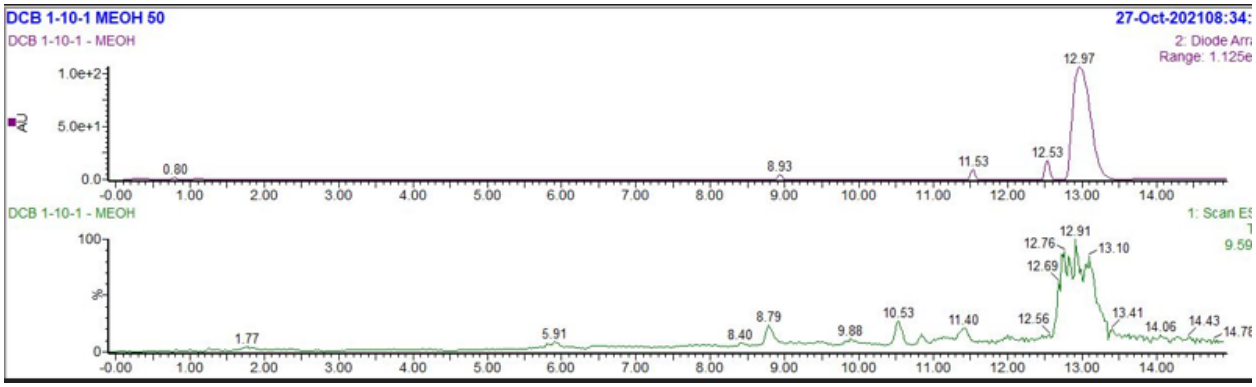
Compound 4 was analyzed by proton NMR and Carbon NMR as well as LCMS (Figure 20). When looking at the LCMS (Figure 20A), we observe multiple peaks around 441 amu which corresponds to the correct predicted molecular weight. The additional peaks around the molecular weight provide further evidence that the aromatic region is attached correctly as the

detector should detect multiple ions with different isotopes of both bromine and chlorine. As for the NMR, we observe the addition of a singlet around 3.5ppm with an integration of 1.89 corresponding to the two protons adjacent to the added aromatic region. We additionally observe an integration of 13.75 in the aromatic region further providing evidence that our predicted structure is present. The carbon NMR also has the addition of the benzyl carbon, a peak located at roughly 40 ppm which we predict from our structure. With all of this data, we can confidently identify our synthetic intermediate as the predicted structure of compound 4.

A.



B.



C.

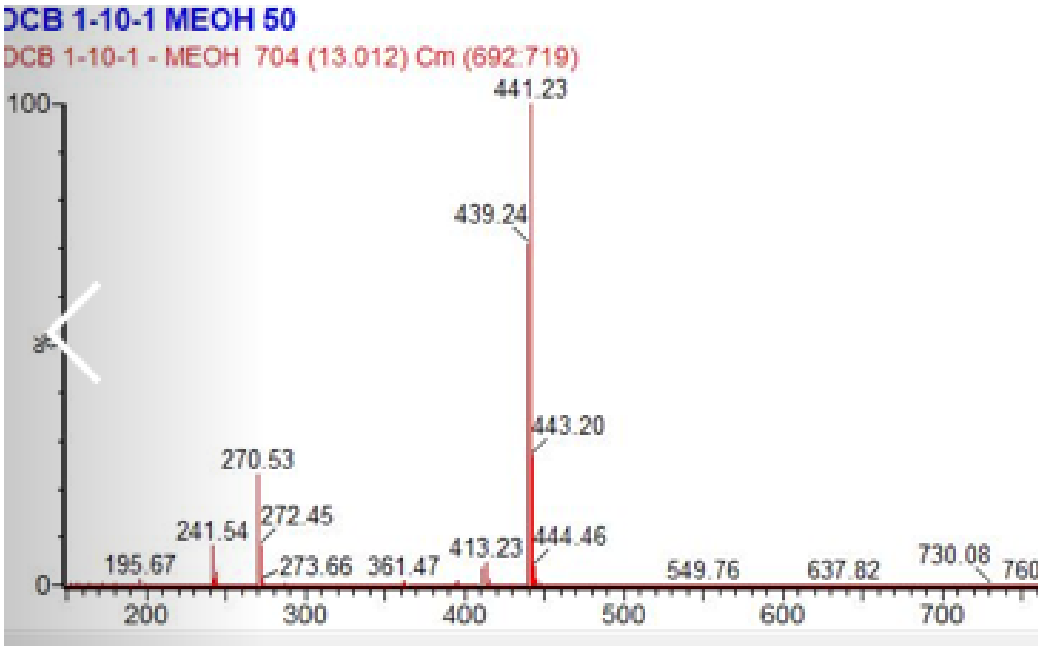
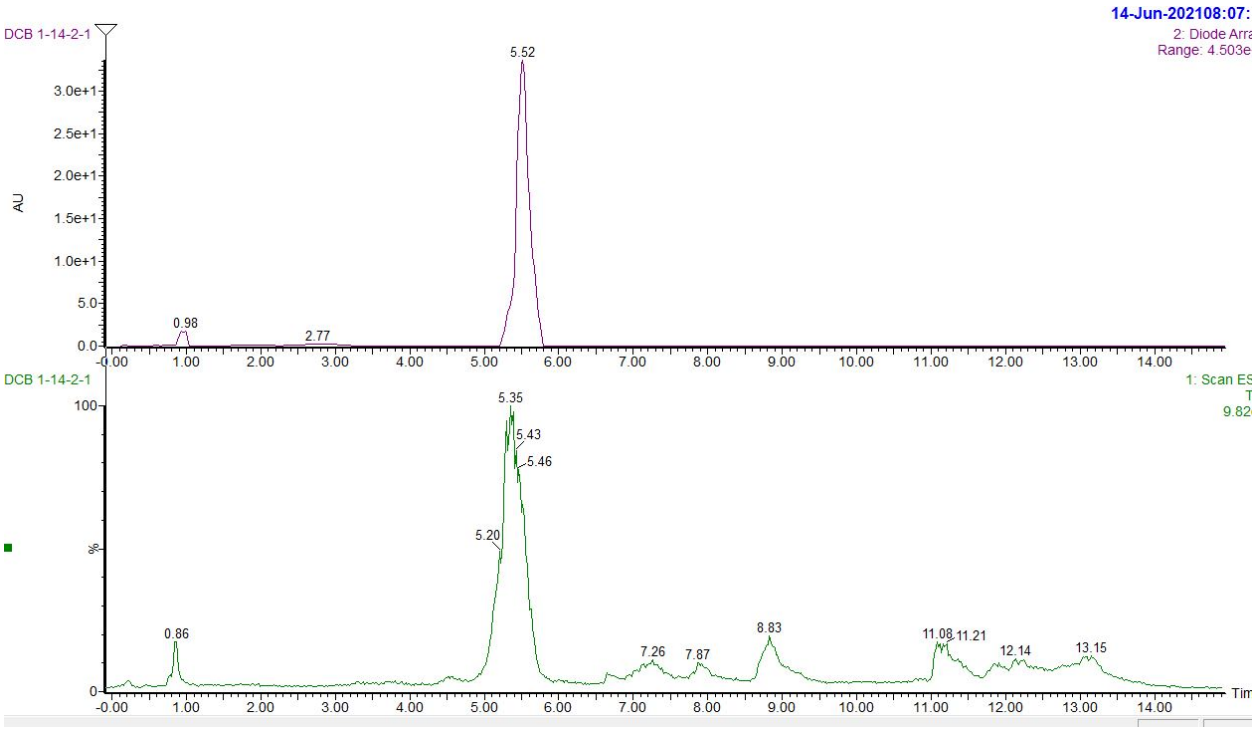


Figure 20: Proton and Carbon NMR of compound 4 in $CDCl_3$. Below is the mass spectroscopy with UV of synthetic intermediate 4.

Once synthetic intermediate 4 was confirmed, a one step synthesis of potentially potent compounds could then be performed. Each synthesis consisted of a simple microwave reaction between 4 and the diamino-hydrocarbon chain being attached at the R2 position. Each crude reaction product was washed with saturated sodium bicarbonate and extracted in diethyl ether. After being run on the preparatory HPLC for purifications, fractions containing the final products were analyzed by LCMS, combined, concentrated and partitioned between saturated sodium bicarbonate and diethyl ether. After evaporation of the diethyl ether confirmatory LCMS was run on final pure samples before biological evaluation.

LCMS of ethyl amine compound 5 showed ions centered around a molecular weight of 455 amu which is in agreement with our predicted molecular weight for the ethyl amine compound (Figure 21). When observing the UV spectrum, we show that our sample is pure as no other peaks were present. Final LCMS was taken after the prep HPLC was performed assuring that impurities were separated and our compound was pure.

A.



B.

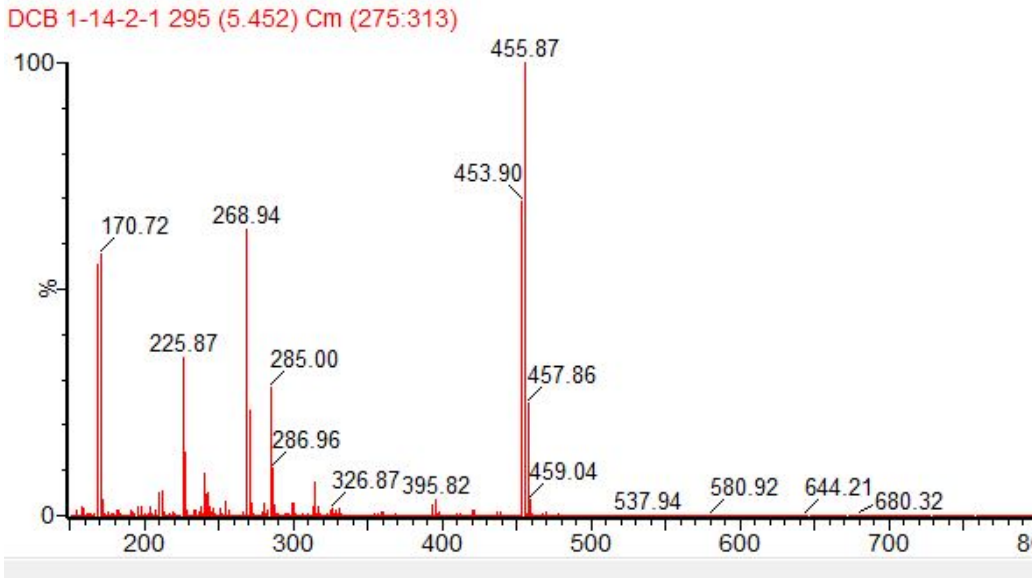
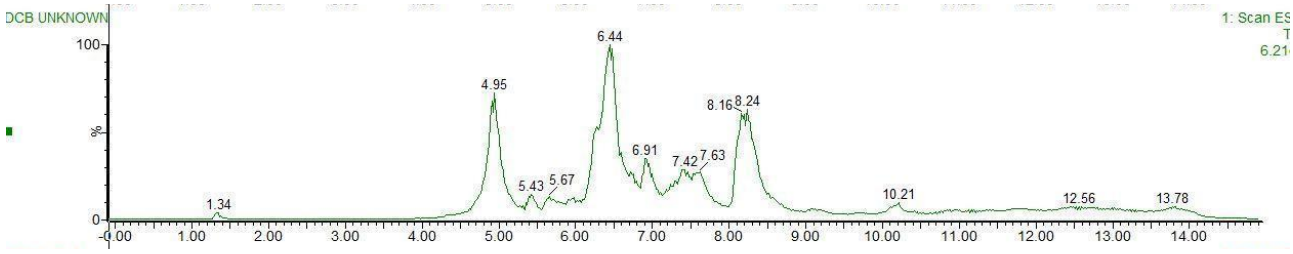


Figure 21: A: Mass spectroscopy and UV spectra of the ethyl compound taken from analytical LCMS. B: Molecular weights of ions present under UV peak from (A)

Analysis of the propyl amine compound 6 was complicated by a malfunctioning UV lamp at the time of synthesis (Figure 22). Mass spectroscopy was still performed to identify the presence of our compound and gave a central peak around the molecular weight of 469. This is exactly 14 mass units above our ethyl amine compound and 14 units below our butyl amine compound corresponding to the molecular weight of a CH_2 being present. Since the reaction and purification process were identical to the ethyl amine and butyl amine compounds, purity was assumed based on the presence of a reasonable mass spectrum. Once UV was restored, an analytical LCMS was rerun on the sample, however the sample had decomposed. Residual

propyl amine compound was still present with the correct molecular weight. An attempt to resynthesize the product was unfortunately unsuccessful due to the microwave not functioning but it is in future plans to resynthesize and retest.

A.



B.

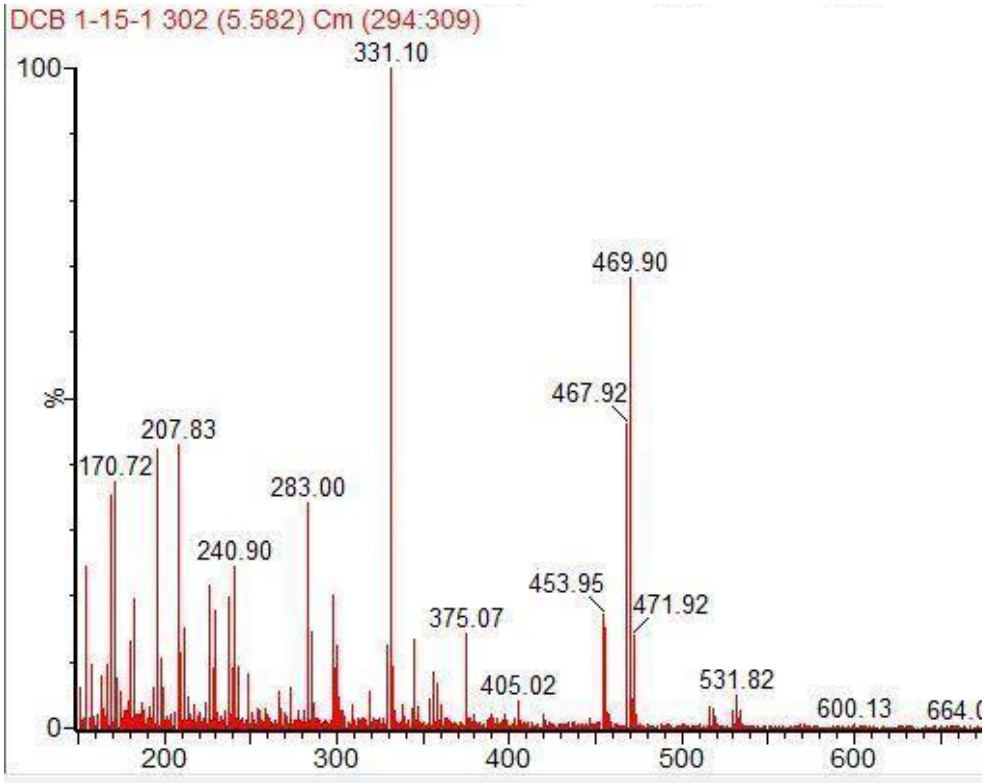
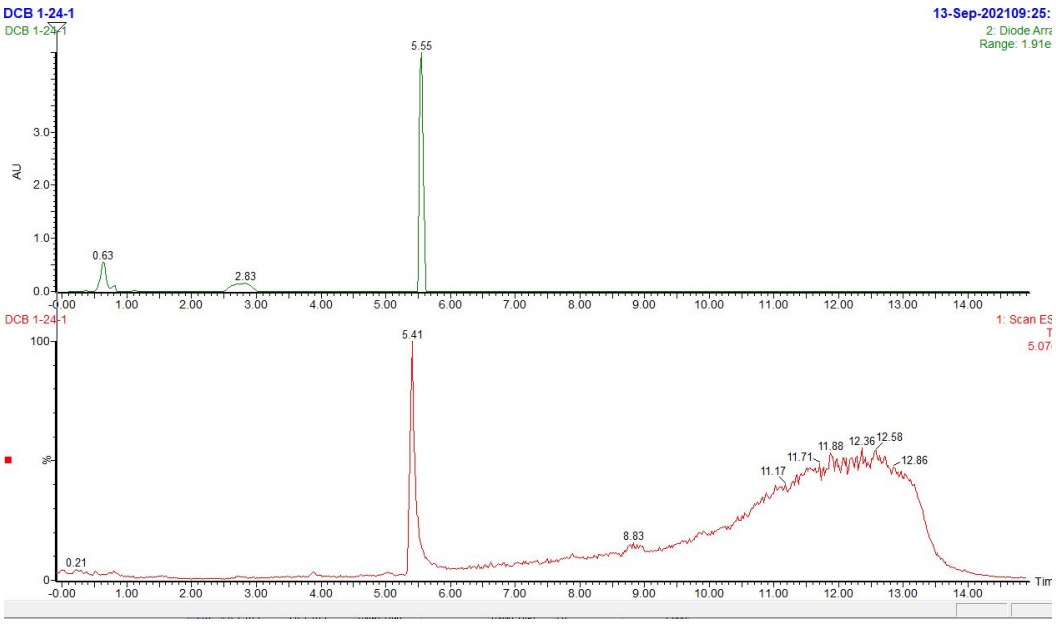


Figure 22: (A.) Spectrum is the UV detection of decomposed propyl amine compounds. (B) Mass spectrum of known propyl amine, compound 5.

Similar analysis to the ethyl amine compound was done for the butyl amine compound 7 (Figure 23). When analyzing the mass spectrum, we observed that the ions correlated with the lone pure UV peak corresponding to a central molecular weight of 483. This corresponds with our predicted structure as the UV spectrum also indicates the purity of the butyl amine compound. When adding two additional methylenes to our ethyl compound we expect an increase in molecular weight by 28, which we observe relative to the ethyl amine compound.

A.



B.

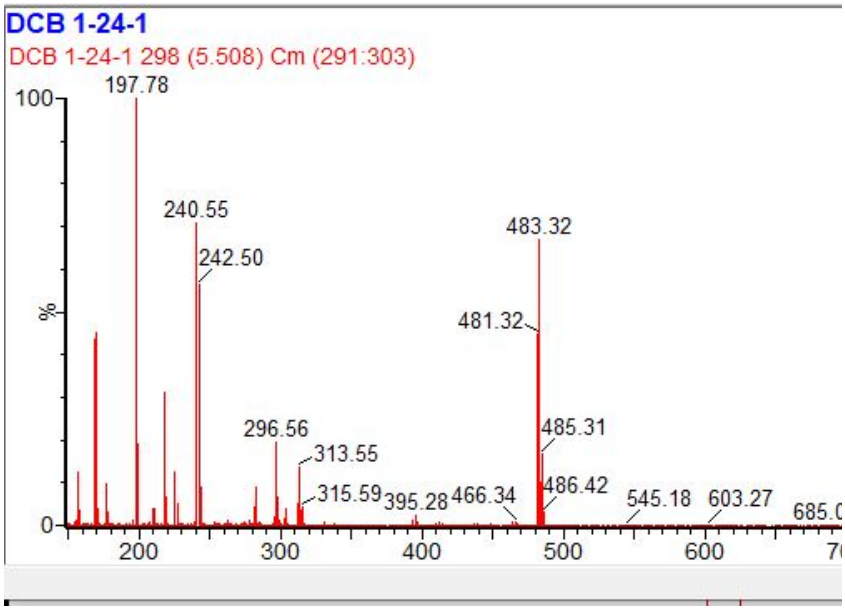


Figure 23: (A:) Mass spectroscopy and UV spectra of butyl compound taken from analytical LCMS. (B:) Molecular weights of ions present under UV peak from (A)

Final compounds were tested with an agar diffusion assay technique. After incubation overnight, the diameter of each zone was measured in millimeters (table 1). Results demonstrated that the amino propyl side chain led to the most potent compound as the zone of inhibition was the largest at every concentration relative to the other compounds produced.

Compound	80µg	40µg	20µg	10µg	5µg	2.5µg
5	15mm	10.5mm	trace	No inhibition	No inhibition	No inhibition
6	19mm	18mm	15mm	12mm	trace	No inhibition
7	13mm	10mm	No inhibition	No inhibition	No inhibition	No inhibition

Table 1: Results of the antimicrobial activity after agar diffusion assay for the three compounds synthesized.

Chapter 12: Discussion

Synthetic intermediate 4 allowed for a 1 step microwave reaction in order to synthesize three amine side groups. By synthesizing this key intermediate, we are able to synthesize multiple products compared to having to complete a full 5 step synthesis for every compound desired. By maintaining the same core structure and optimizing the R2 side chain, we succeeded in synthesizing the most potent antibacterial to date in our current series. Future modifications in other regions of the molecule will be explored while maintaining the propyl amine side chain.

Confirmation of all synthesized structures was important in the overall synthetic route. Analytical tools , such as LCMS and NMR were employed to confirm. The synthesis steps went according to plan. By performing the LCMS of each final product, strong evidence of a pure compound with the correct molecular weight was determined. Confirming each synthetic product along the way was important in order to allow for the subsequent reactions to be performed.

Ethyl, propyl, and butyl amine analogues were chosen in order to test whether the length of the amine group at the R2 position had a direct effect on antibacterial potency. In order to

confirm that each compound had only a difference in a methylene, mass spectra data can be compiled to show a difference in 14 amu between each compound. As the data in table 1 demonstrates, the size of each amine side chain had a clear effect on antibacterial potency when the bioassay was run. The propyl compound hit a sweet spot in length. Over a four fold decrease in potency was observed by subtracting or adding a simple methylene to each side chain. Computational chemistry produced a model that predicted the amine interacting with the Ca^{2+} in FtsZ.[39] Since amines are basic, the negative charge will be attracted to the positive calcium ion creating a strong binding interaction of our compound to the FtsZ protein allowing for better inhibition.

Agar diffusion assays were run to test the potency against *S. aureus*. Although our goal is to develop compounds that inhibit methicillin resistant *S. aureus*, this microorganism cannot be tested at Drew. We can infer that since the mechanism of action is through inhibition of a novel molecular target, FtsZ, it is unlikely that significant resistance already exists in the clinical setting since mutant FtsZ is not likely favorable for survival.

Chapter 13: Conclusion

On a biochemical level, computational chemistry predicted that our compounds bind to the interdomain cleft on FtsZ.[39] Analyzing the crystal structure of FtsZ, a calcium ion is located in this region of the protein which is essential for protein interactions (Figure 24). Since the propyl amine compound is the most potent, the potential alignment of the amino group to the calcium leads to the strongest interaction observed at this time. .

The overall findings were that the propyl amine compound had the highest potency against *S. aureus* bacteria when tested compared to the ethyl and butyl amine compounds.

However, the potency determined by the zone of inhibition was much less than the control ciprofloxacin which is a marketed antibiotic. Modifications of the isoindole are needed in order to further increase potency. Future work includes additional modifications beyond simple amines at the R2 location introducing functional groups such as amides, sulfonamides, and sulfhydryls.

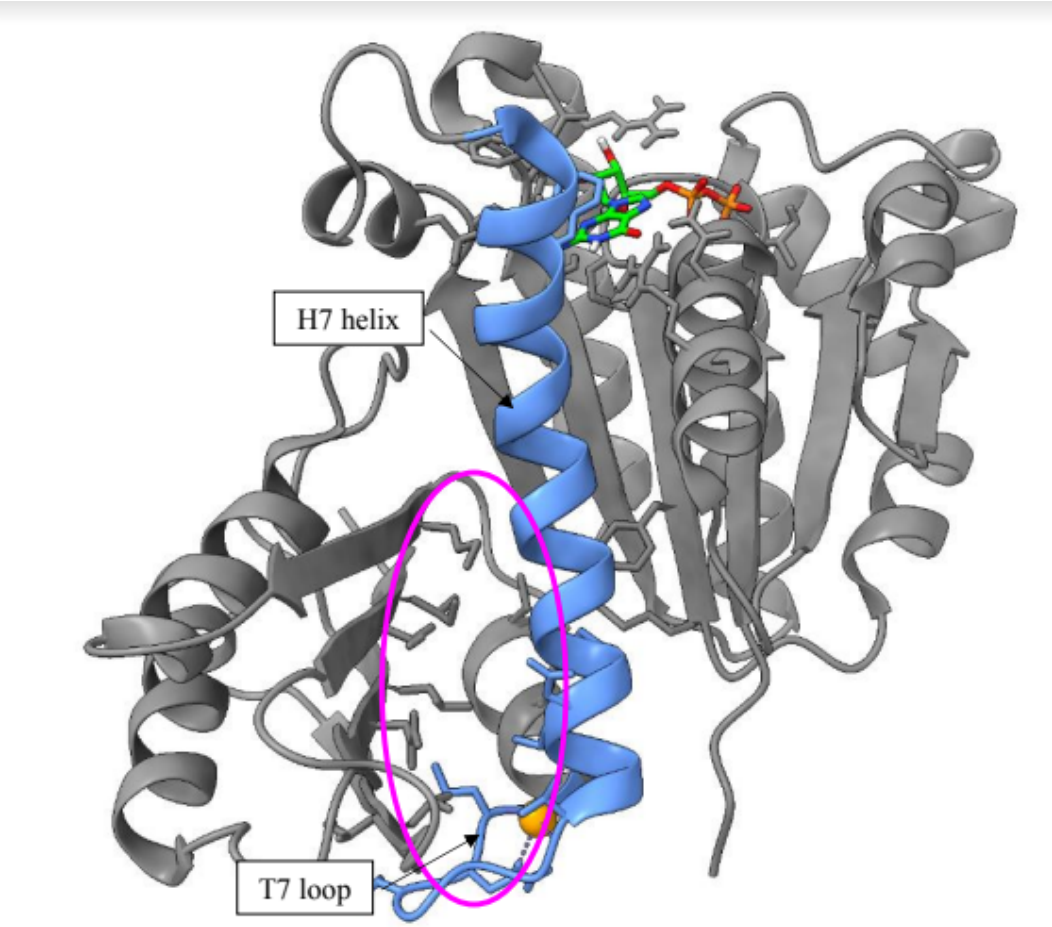


Figure 24: FTSZ crystal structure identified via x-ray crystallography. The highlighted pink circle is where our compounds are predicted to bind to. In orange, the Ca^{2+} is where our R2 chain is predicted to chelate.[39.]

During this research, only potency of our compound was addressed while other pharmacokinetic properties were neglected. In order for a compound to eventually become a drug, both potency and pharmacokinetics must be optimized before continuing in the drug discovery process. The lipophilicity of these compounds are too high to achieve good pharmacokinetics since we have three aromatic rings in our final structure which are very hydrophobic. We also have not tested whether our compound is orally absorbed, or interacts with any metabolizing enzymes. No pharmacokinetic data has been collected that addresses the ADME properties. Future students intend to continue synthesizing analogues to address potency and pharmacokinetic properties. If antibiotic resistance remains unaddressed, a projected 10 million people will die annually by 2050 from antimicrobial resistant infections.[41] This would be predicted to be the leading cause of death worldwide.

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